# DIETS OF FOUR EELPOUT SPECIES (GENUS *LYCODES*) IN THE U.S. BEAUFORT SEA BASED ON ANALYSES OF STOMACH CONTENTS AND STABLE ISOTOPES OF NITROGEN AND CARBON

By

Sarah J. Apsens, B.S.

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APPROVED:

Brenda Norcross, Committee Chair
Katrin Iken, Committee Member
Franz Mueter, Committee Member
Andrés López, Committee Member
Milo Adkison, Chair
*Department of Fisheries*
S. Bradley Moran, Dean
*College of Fisheries and Ocean Science*
Michael Castellini, *Dean of Graduate School*

**Abstract**

Eelpouts of the genus *Lycodes* are an abundant group of demersal fishes in the U.S. Beaufort Sea. Currently eelpout diet and the exact role of eelpouts in the Arctic food web are poorly understood. Additionally, if and how eelpouts avoid intra- and interspecific competition for resources is unknown. In this study, diets of four common Beaufort Sea eelpout species were analyzed with respect to along-shelf (longitude) gradients, across-shelf (depth) gradients, and ontogeny (fish body length) to determine diet composition and patterns of resource partitioning. Diets of the four most numerous eelpout species were analyzed using a combination of stomach contents and nitrogen and carbon stable isotope analyses: Adolf's Eelpout *Lycodes adolfi,* Canadian Eelpout *L. polaris,* Archers Eelpout *L. sagittarius,* and Longear Eelpout *L. seminudus*. Nitrogen stable isotopes of fish tissue were analyzed to determine trophic level and carbon stable isotopes to determine if origin sources of carbon in food web pathways of eelpout diets differed among species. Fishes were collected in the central (2012) and eastern (2013 and 2014) Beaufort Sea in August and September as part of the U.S.-Canada Transboundary program. Prey groups Polychaeta, Amphipoda, Isopoda, Ophiuroidea, and Copepoda composed a large proportion of the diet by percent weight for all four species of *Lycodes*, but their relative contributions differed among the species examined. This study indicated that eelpouts feed almost exclusively on benthic prey and avoid interspecific competition by occupying different habitat space and having different diets. Intraspecific similarity in diet composition was low suggesting these fish have diverse diets even among individuals of the same species. Fish length was associated with changes in diet composition for *L. adolfi* and *L. sagittarius*, but not *L. polaris* and *L. seminudus*. Longitude and depth were correlated with shifts in diet composition for *L. sagittarius*, but not the other three species. *Lycodes polaris* occupied a lower trophic level than the other three eelpout species based on nitrogen stable isotope values. Despite differences in the across-shelf distribution between *L. polaris* and the three deep-water eelpout species, carbon sources of diet were indistinguishable among the four eelpout species. Ecological information on abundant Arctic fish species like eelpouts is needed for long-term ecosystem monitoring, which is especially important in light of pronounced climate changes and increased human activities in the Arctic.

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## Introduction

Arctic fishes are important links between lower and upper trophic levels (Lowry and Frost 1981). Fish consume plankton, benthic invertebrates, and smaller fishes. These fish are then available to higher trophic level organisms like birds, whales, ice seals, polar bears, and humans. Basic ecological information on abundant but poorly studied fish species is needed as state and federal agencies prepare for multispecies management practices in the Arctic, such as the potential development of commercial fisheries (NPFMC 2009). Here, I provide new insights into the diet and trophic ecology of four abundant eelpout species thus providing a valuable benchmark for long-term, multispecies ecosystem monitoring in a changing Arctic.

Understanding the current statuses and processes of the abiotic and biotic components of the Arctic ecosystem is becoming increasingly urgent under unprecedented environmental change (Linden 2016; IPCC 2014). Climate change is expected to alter Arctic Ocean ice cover, which in turn will impact existing patterns of primary production, which will reverberate throughout the associated food web (Carmack and Macdonald 2002; Bluhm and Gradinger 2008; Grebmeier 2012). An ecosystem-wide shift is expected, resulting in higher transfer of organic carbon to pelagic consumers rather than to benthic communities (Grebmeier et al. 2006). Understanding the role of abundant organisms that form essential ecological links in the current Arctic food web is necessary to better predict changes to how the Arctic ecosystem currently functions.

Zoarcidae is a large and species-rich family of fishes commonly known as eelpouts. Approximately 240 species in this family are recognized globally (Anderson and Fedorov 2004). Eelpouts are found in both the Arctic and Antarctic seas and in boreal regions across both hemispheres, usually in deep waters off continental shelves (Anderson 1988; Mecklenburg et al. 2011). In the Arctic, eelpouts are circumpolar in distribution and primarily represented by two genera: *Lycodes* and *Gymnelus* (Mecklenburg et al. 2011). *Lycodes* is the more species-rich genus of the two, and includes 24 of the 34 known Arctic species in the family Zoarcidae (Møller and Gravlund 2003; Mecklenburg et al. 2011). In addition to being species rich, Zoarcidae is one of the most abundant demersal fish families in the U.S. Beaufort Sea, superseded only by the families Gadidae and Cottidae (Rand and Logerwell 2011; Giraldo et al. 2016; Norcross et al. 2016). Approximately 13 fish families are represented in the central and eastern U.S. Beaufort Sea (Norcross et al. 2016). The shelf ($\leq$ 100 m) is dominated by Gadidae and Cottidae, but on the central and eastern Beaufort Sea slope ($\geq$ 200 m in depth) zoarcids of the genus *Lycodes* compose over half of the total fish

biomass (>60%) and abundance (>60% in 2013 and52% in 2014) (Norcross et al. 2016). Due to their abundance, eelpouts may be an important component of the ecosystem, potentially competing with other fish for resources, serving as prey themselves, and/or actively preying on other fish species (Møller and Jørgensen 2000). Despite their potential ecological importance in the Arctic Ocean and adjacent seas, surprisingly little is known about zoarcid diet and trophic position in the western Arctic.

Similar to other Arctic fish species, eelpouts serve as prey for higher trophic level Arctic organisms. Seabirds like Black-Legged Kittiwakes *Rissa tridactyla* and Northern Fulmars *Fulmarus glacialls* occasionally consume *Lycodes* spp. (Phillips et al. 1999; Paredes et al. 2014). Marine mammals, including bearded seals *Erignathus barbatus* and belugas *Delphinapterus leucas* consume eelpouts in the Bering and Chukchi seas (Lowry et al. 1980; Finley and Evans 1983; Quakenbush et al. 2015). Greenland Shark *Somniosus microcephalus* in the Atlantic Arctic consume eelpouts (Yano et al. 2007). Eelpouts are occasionally consumed elsewhere by humans (Love 2011), but they are not used for subsistence in the Pacific Arctic. Due to the lack of commercial fishing for eelpouts globally, the development of commercial fishing for Arctic eelpouts is unlikely; however, eelpouts could be bycatch if commercial fisheries were to develop in the region.

Eelpouts of the genus *Lycodes* have relatively long lifespans compared with other fishes in the region. For example, the Glacial Eelpout (*Lycodes frigidus*) is thought to achieve a maximum age of 33 years, while other eelpouts species likely reach maximum ages between 6 and 24 years (Balanov et al. 2006; Hildebrandt et al. 2011; Norcross et al. 2016). In contrast, the most abundant Arctic forage fish, Arctic Cod *Boreogadus saida*, only lives 5 to 8 years in the Beaufort Sea (Gillispie et al. 1997; Frothingham in progress). Due to their relatively long lifespans, eelpout populations may respond more slowly phenotypically to environmental perturbations than organisms with shorter generation times and thus more adaptive potential (Davis et al. 2005; Somero 2009). Eelpouts may be susceptible to bioaccumulation of toxins due to their long lifespans. Mercury (Atwell et al. 1998) and persistent organochlorine (OC) contaminants (Borgå et al. 2004) are shown to bioaccumulate in some long-lived members of the Arctic food web. Microplastics have been observed in high concentrations in Arctic Sea ice (Obbard et al. 2014), and consumption of released microplastics could expose fishes to physiological stress and toxins that could accumulate in tissues (Rochman et al. 2013).

The eelpout species examined in this study are the most numerous of the zoarcids collected as part of a joint U.S. and Canada effort to document fish and invertebrate species in the Beaufort Sea called the U.S.-

Canada Transboundary Fish and Lower Trophic Communities project; the eelpout species have overlapping distribution ranges in the Beaufort Sea (Norcross et al. 2016). *Lycodes adolfi* is typically found in high numbers between 800 and 1,200 m deep off Greenland and Norway (Møller and Jørgensen 2000; Byrkjedal et al. 2011), and only recently was discovered to occupy the western Arctic (Mecklenburg et al. 2011). It spawns in the summer, while most other Arctic eelpout species spawn in late fall or winter (Møller and Jørgensen 2000). *Lycodes sagittarius* and *L. seminudus*, found from the Beaufort Sea to the Kara Sea, commonly occur on the slope (> 100 m) on muddy substrates (McAllister et al. 1981). *L. polaris* is circumpolar in distribution (Mecklenburg et al. 2002) and is found in both marine and brackish nearshore waters (Craig 1984) at shallower depths than the other three *Lycodes* species in this study (Norcross et al. 2016). The abundance and potential niche overlap of the four eelpout species in the Beaufort Sea warrants further investigation of their diet and trophic roles to address questions of competition and resource partitioning.

Diet information is limited for the four eelpout species examined here. In general, eelpouts are demersal, and all *Lycodes* have cartilaginous stationary crests on their chins that are believed to be used to skid through the sediment while looking for prey (Anderson 1994). *Lycodes polaris* in the Chukchi Sea feed heavily on demersal, gammarid amphipods (Whitehouse et al. 2017). In contrast, *L. polaris* in the neighboring Beaufort Sea may have a more pelagic-based diet, and is characterized as a low-trophic position generalist (Giraldo et al. 2016). The differences in diet composition for *L. polaris* between seas enforces the need for regional diet studies such as this one. *Lycodes adolfi* diet in the Canadian Beaufort Sea consists of demersal prey (Giraldo et al. 2016) but is highly variable among individuals. *Lycodes sagittarius* stomachs collected in the far eastern Beaufort Sea contained annelid worms, mollusks, and crustaceans; the presence of vomerine teeth often used for crushing prey in other fish species suggest that *L. sagittarius* may specialize in preying on hard-shelled prey (McAllister et al. 1981). *Lycodes seminudus* is characterized as a mid- to high-trophic level benthic generalist, potentially feeding on overwintering *Calanus* spp. copepods(Giraldo et al. 2016). Amphipods, decapods, isopods, and polychaetes have been observed in *L. seminudus* stomachs collected in the Barents Sea (McAllister et al. 1981). Fatty acid and stable isotope signatures consistent with a diet of *Calanus* copepods were observed for *L. adolfi* and *L. seminudus* collected in the Canadian Beaufort Sea, suggesting copepods like *Calanus hyperboreus* may be important diet components for the two eelpout species (Giraldo et al. 2016).

Two common ways to study diet are stomach content analysis and stable isotope analysis, which together provide complementary information about trophic ecology. Analyzing stomach contents provides high

taxonomic resolution of prey species as well as abundance (e.g., counts) or size of prey (Pinkas et al. 1971; Hyslop 1980). Reliable prey identification during stomach content analysis, however, can be biased towards prey items with hard, indigestible body parts (Baker et al. 2014). Prey organisms with soft bodies, like polychaete worms, are digested rapidly compared with hard-bodied prey. Therefore, while stomach content analysis gives a detailed taxonomic account of a consumer's diet, this information represents a short time period, i.e., hours or days after consumption, and is biased toward prey that have identifiable hard structures. Application of DNA to identify heavily digested prey in fishes (Dunn et al. 2010) can be useful in stomachs with highly digested prey, but has drawbacks including cost and the biases introduced in the application of the need for prey species-specific primers (Jarman et al. 2004). Stable nitrogen isotope analysis can give time-integrated information on diet and relative trophic level of a species based on feeding strategies, but does not provide information on specific prey composition without additional ancillary data (i.e., stable isotope values of prey consumed) (Vander Zanden and Rasmussen 1999; Kelly 2000). Ultimate carbon sources from pelagic, sea-ice associated, and terrestrial production can be distinguished isotopically (Iken et al. 2005; Dunton et al. 2006; Gradinger 2009; Bell et al. 2016). Stable isotope ratios integrate fish diet information over weeks to possibly even months (Sakano et al. 2005; Buchheister and Latour 2010). Therefore, stable isotope analysis complements taxonomically-detailed results from stomach content analysis with a broader temporal picture of the trophic ecology of *Lycodes* species.

Physical features of a habitat, e.g., water mass characteristics and depth, can influence epibenthic prey distribution (Ravelo et al. 2015) and potentially the diet composition of predators like fish or large invertebrates (Fahrig et al. 1993; Jaworski and Ragnarsson 2006; Divine et al. 2015). In this study, fish were collected across the shelf and the slope habitats of the Beaufort Sea. The steep slope cuts through multiple, layered water masses that create different environments based on salinity, temperature and nutrient regimes (Pickart et al. 2011). The changes in water masses across depth are closely linked to changes in benthic infauna and epifauna communities that can serve as prey for eelpouts (Nephin et al. 2014; Roy et al. 2015). Terrestrial organic matter input from major rivers also results in longitudinal differences in trophic structure, carbon isotopic signatures, and benthic invertebrate food web length on the Beaufort shelf (Bell et al. 2016). Regional variation in trophic structure and carbon sources as shown for invertebrates (Divine et al. 2015; Bell et al. 2016) may also be reflected in eelpouts.

Intra- and interspecific interactions among fishes influence diet composition (Chipps and Garvey 2007). Fishes that share the same habitat and trophic level often compete for resources (Parish 1975). Eelpouts

consume epifauna (Bjelland et al. 2000; Dissen 2015; Giraldo et al. 2016). Epifauna biomass is greater than fish biomass in the U.S. Beaufort Sea, is highest at the shelf break, and decreases with increasing depth (Norcross et al. 2016). Although epifauna prey availability may not be a limiting factor, the four eelpouts species in this study could be competing for the same resources if they share the same trophic level and habitat unless resource partitioning is occurring. Decreasing epifauna abundance with increasing depth may increase inter- and intraspecies competition for resources. Resource partitioning among sympatric Arctic fishes of the order Scorpaeniformes that share habitat space has been observed in northern Norway (Källgren et al. 2015) and in the Beaufort Sea (Gray et al. 2017). Eelpouts may also interact with other demersal fish species either through competition or predation. Some evidence for interspecific interaction exists. In the Canadian Beaufort Sea, *L. polaris* diet overlaps with Arctic Staghorn Sculpin (*Gymnocanthus tricuspis*), another abundant demersal fish species (Giraldo et al. 2016). Examining diet of these four eelpout species will elucidate patterns of resource partitioning or diet overlap.

In order to distinguish the intraspecific diet and trophic roles of eelpouts in the ecosystem it is necessary first to have a robust understanding of species and population divisions. Eelpout species' boundaries and taxonomic descriptions have traditionally been based on morphological features (Anderson 1994; Møller and Gravlund 2003). However, the extensive phenotypic variability with size and sex documented in some species of *Lycodes* points to potential problems with current taxonomic designations (McAllister et al. 1981; Møller and Jørgensen 2000; Balanov and Kukhlevskii 2011; Mecklenburg et al. 2011). For example, one eelpout species from the Sea of Japan occurs in five different major color variations (Balanov and Kukhlevskii 2011). Spatial differences in color patterns at the population level have also been described for *L. seminudus* (Møller and Jørgensen 2000; Mecklenburg et al. 2014), and polymorphic populations of *Lycodes* exist in the Northern Hemisphere (Anderson and Fedorov 2004). Genetic analysis using mitochondrial DNA (mtDNA) is one method commonly used to assign individuals to species when morphological characteristics are not reliable, though this approach has limitations. MtDNA analysis has been used to clarify identification of the Arctic eelpout species *L. yamatoi* (Balanov and Kukhlevskii 2011) and the overall structure of Arctic eelpout phylogeny (Møller and Gravlund 2003) and diversity (Turanov et al. 2016). An important limitation of mtDNA is that it only provides a partial description of genetic variability entirely restricted to the mitochondrial genome; in addition, it is susceptible to error in populations where hybridization occurs (Ward et al. 2005). Though not a conclusive measure in itself mtDNA can aid in identifying individuals when other methods are not reliable. Sequences from mtDNA

were employed in the present project to ensure that results on diet, trophic position, and potential resource partitioning or competition were placed in the appropriate species context.

The objective of this study was to characterize diet of *L. adolfi*, *L. polaris*, *L. sagittarius*, and *L. seminudus*, four common eelpout species on the Beaufort Sea shelf and slope, and to look for evidence of resource partitioning. To accomplish this, I described the diet and inferred trophic level (TL) using stomach content and stable nitrogen and carbon isotope analyses. Sampling over three years afforded the opportunity to evaluate interannual variability. In addition, stomach contents and stable isotopes were compared across fish length to see if resource partitioning changes through eelpout ontogeny, and by depth to test for resource partitioning by habitat. Detailed information on diet is needed for abundant fish species such as eelpouts to better understand patterns of resource use and partitioning and to inform ecological models for the Arctic (Whitehouse et al. 2017; Källgren et al. 2015). This type of information is also needed for understanding current ecosystem functioning and for establishing baseline information required for long-term monitoring of the ecosystem.

## Methods

*Fish collection and processing*

Eelpouts were collected over three years during the U.S.-Canada Transboundary cruises in the central and eastern Beaufort Sea (Figure 1). The central U.S. Beaufort Sea was sampled in 2012 (September 20 to October 1, between 151.5° – 150.5° W and 70.5° – 72° N), and the eastern Beaufort Sea in 2013 (August 12 to September 2, 146.1° – 136.7° W, 70° – 72°N) and 2014 (August 17 to September 2, 146.1° – 140.1° W, 70° – 72°N). Sampling occurred on eleven predetermined across-shelf transects (approximately following lines of longitude) and along-shelf, at depths of 20, 50, 100, 200, 350, 500, 750, and 1,000 m, except for 2014 when also one 1,500 m station was sampled. An otter trawl (38 mm mesh in body), and three beam trawls (BT; 7 – 10 mm mesh in body and 4 – 6 mm mesh in codend) were used to collect fish (for description of nets see Norcross et al. 2016). All nets were towed at 1–2 kts for approximately 3–15 min. Total time the net was on the bottom was determined from a Star-Oddi time depth recorder (TDR) attached to the net, and haul distance was calculated from GPS locations of the vessel at the start and end time of the net on the bottom. Effort was comparable for the three BT nets (Norcross et al. 2016). Catch per unit effort (CPUE) was calculated for eastern Beaufort Sea (2013 and 2014) BT catches as (# fish x 1,000) / (haul distance (m) x 2.26 m net swath). CPUE was not calculated for central Beaufort Sea (2012) and OT catches because swaths were inconsistent and equipment issues resulted in unreliable haul information. Fishes from both regions (central and eastern) and all net types were used in subsequent stomach content and stable isotope analyses.

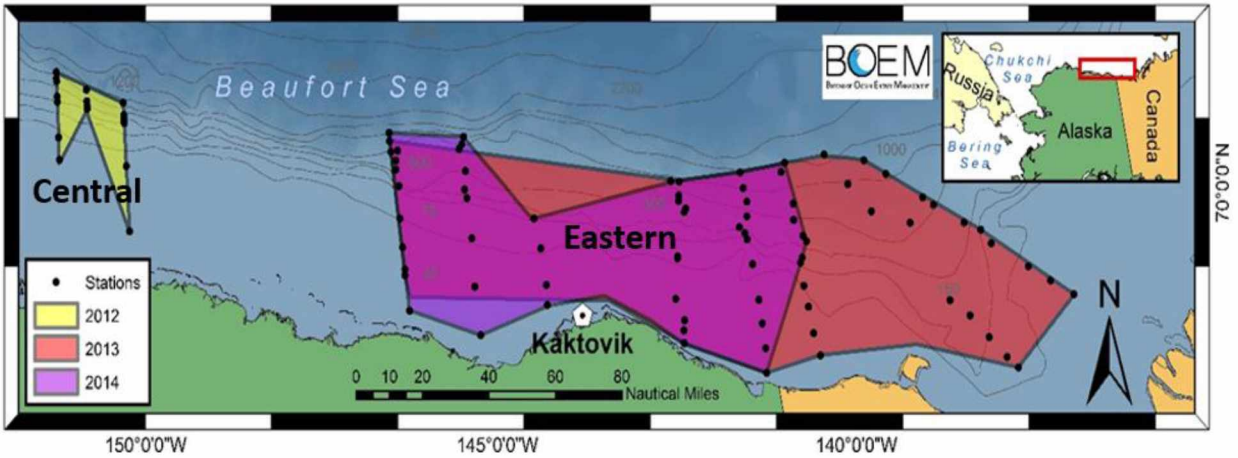


Figure 1. Area sampled. Sampling stations (black dots) occurred along transects oriented perpendicular to shore. Polygons cover areas sampled in each year.

Eelpouts obtained at sea were identified, euthanized, and frozen for later processing. Eelpouts were identified to species when possible or to the genus or family level when they were too small or damaged to identify accurately in the field. A lethal dose of MS-222 was used to euthanize the fish (Institutional Animal Care and Use Committee protocol #07–047). Eelpouts were then frozen in the field for later processing at the Fisheries Oceanography Lab (FOL) at the University of Alaska Fairbanks.

At FOL morphometric measurements and tissue samples were collected. Wet weight to the nearest 0.01 g and total length (mm) of each fish were measured before the stomach was removed. Whole stomachs were removed from each eelpout by making an incision on the ventral side and cutting at the esophagus and pyloric valve and stomachs were frozen in water. In total, stomachs from 466 eelpout specimens were examined from the three cruises (Table 1). Gape height to the nearest 0.01 mm was measured from the top of the dentary to the bottom of the premaxilla using digital calipers while the mouth was at maximum extension (Scharf et al. 2000). Gape height was only measured for the four eelpouts collected in 2014 (n=184). Fishes from 2012 and 2013 were not measured for gape size because repeated freezing and thawing may have compromised gape morphology. Fish with broken jaws or fish whose jaws were too small for accurate measurement were not measured.

Table 1. Number of fish stomachs by eelpout species and sampling year. Excluded were those stomachs that were empty, non-quantitative (burst stomachs), or contained only parasites.

| Species, Cruise | Available | Excluded | Empty | Parasites | Burst | Used in Analysis |
|---|---|---|---|---|---|---|
| *Lycodes adolfi* | 164 | 41 | 39 | 1 | 1 | 123 |
| 2012 | 25 | 9 | 9 | 0 | 0 | 16 |
| 2013 | 47 | 9 | 9 | 0 | 0 | 38 |
| 2014 | 92 | 23 | 21 | 1 | 1 | 69 |
| *Lycodes polaris* | 44 | 10 | 10 | 0 | 0 | 34 |
| 2012 | 30 | 5 | 5 | 0 | 0 | 25 |
| 2013 | 1 | 1 | 1 | 0 | 0 | 0 |
| 2014 | 13 | 4 | 4 | 0 | 0 | 9 |
| *Lycodes sagittarius* | 151 | 21 | 18 | 3 | 0 | 130 |
| 2012 | 75 | 12 | 10 | 2 | 0 | 63 |
| 2013 | 18 | 2 | 2 | 0 | 0 | 16 |
| 2014 | 58 | 7 | 6 | 1 | 0 | 51 |
| *Lycodes seminudus* | 107 | 25 | 16 | 9 | 0 | 82 |
| 2012 | 39 | 7 | 5 | 2 | 0 | 32 |
| 2013 | 33 | 12 | 6 | 6 | 0 | 21 |
| 2014 | 35 | 6 | 5 | 1 | 0 | 29 |
| **TOTAL** | **466** | **97** | **83** | **13** | **13** | **369** |

*Species identification*

Consultation with an Arctic fish taxonomist (C.W. Mecklenburg, Point Stephens Research, Auke Bay, AK) revealed difficulties with accurately identifying specimens of *Lycodes* to species. Because morphological re-identification was impossible after individuals had been cut up during processing, mtDNA barcoding was used to aid in fish species identification. DNA isolated from muscle samples from 205 specimens was used to determine DNA sequences from a segment of the mitochondrial *cox1* gene (commonly termed the DNA barcode). Briefly, total genomic DNA was isolated from frozen tissue samples using standard molecular biology protocols. Genomic DNA preparations were then used as templates in amplification reactions using the Fish F1/R1 primer set of Ward et al. (2005). Reaction products were purified and sequenced in both directions using the Sanger protocol. Raw sequencing data were reviewed, edited, and assembled to exclude artifacts introduced during sequence determination procedures. Finished sequences were used in match queries against the Barcode of Life Database (BOLD; *barcodeoflife.org*) to determine their species assignations. A match was deemed acceptable when query sequences from this study matched published sequences from vouchered specimens at levels of >99.0%.

In cases where sequences representing multiple species in BOLD matched a sequence from the study specimen, the individual fish was deemed to belong to a species complex. Here, a species complex is defined as a group of closely related species, or species that show very little genetic differentiation based on the barcode gene. BOLD results were compared to the previous species identifications based on morphology. In total, 204 fishes originally identified by FOL as one of the four species of *Lycodes* examined were successfully sequenced for mtDNA, and an additional 40 minimally processed and voucher specimens were identified based on morphological features by the expert taxonomist. The estimated agreement between molecular and morphometric methods, here referred to more generally as percent accuracy, in identification for each eelpout species was determined by dividing the number of fishes identified correctly by FOL by the total number of fishes identified to species by mtDNA or an expert taxonomist.

*Stomach Content Identification*

Each stomach was thawed and opened under a dissecting microscope (6x to 100x magnification). Prey were identified to the lowest taxonomic level possible, length measured (mm) and weighed to the nearest 0.0001 g. Heavily digested prey were designated as either unidentified crustacean carapace fragments or as other unidentified animal soft tissue. In total, stomachs of 369 individual fish were analyzed; 97 stomachs were excluded because they were empty, non-quantitative (burst stomachs), or contained only parasites (Table 1).

Individual prey taxa were clustered into coarse prey groups to ensure adequate description of eelpout diet. Prey were initially identified to the lowest taxonomic level possible. Prey-accumulation curves were used to assess how adequately diet was described at fine-scale taxonomic levels. A species' diet was considered adequately described when the prey-accumulation curve reached an asymptote (Chipps and Garvey 2007). Asymptotes were not achieved with prey identified to the lowest taxonomic level possible, indicating eelpout diet was not sufficiently described, so prey were aggregated into coarse taxonomic groups at either phylum, order, class, or subclass level. Rare prey were grouped as "other". A prey item was considered rare if it occurred fewer than five times across all stomachs analyzed across all four eelpout species. All prey-accumulation plots were created using bootstrapping with 999 permutations as implemented in PRIMER v.7.

Percent wet weight (%W) of prey was chosen to describe diet for each eelpout species because of its potential ecological significance. This index can be indicative of the nutritional importance of a prey item

(Hyslop 1980, Macdonald and Green 1983, Chipps and Garvey 2007) and was calculated for each prey group *i* and predator stomach *j* as follows:

$$\%W_{ij} = \frac{W_{ij}}{\sum_i W_{ij}} * 100$$

where $W_{ij}$ is the weight (g) of all members in a prey group *i* in the stomach of a predator *j*, divided by the sum of all prey group weights in the stomach of predator *j*.

An additional index, percent mean weight (%MW), was used to describe overall diet composition for each eelpout species by averaging $\%W_{ij}$ over all individual stomachs *j*:

$$\%MW_i = \frac{1}{P}\sum_{j=1}^{P}[\%W_{ij}]$$

where *P* is the total number of non-empty stomachs.

*Interannual Differences in Diet*

A permutational analysis of variance (PERMANOVA, PRIMER v.7) based on Bray-Curtis dissimilarity matrices using %W was used to test for interannual differences in diet composition for fishes collected in 2013 and 2014 (eastern Beaufort Sea). Sampling year was used as a fixed factor, and tests between years were run separately for each eelpout species. Significance level was set at $\alpha = 0.05$. If diet did not differ between years for a given eelpout species then diet data were pooled across years. Cumulative prey curves of each eelpout species in each sampling year were used to see if pooled data were necessary to more comprehensively describe diet. Because of the different sampling area, 2012 fish diet was analyzed separately to avoid confounding effects between space and time (Figure 1).

*Gape size*

Analysis of covariance (ANCOVA) models were used to determine the relationship between fish length and gape height among the four eelpout species. The relationship between fish length and gape height is linear (Scharf et al. 2000), but three alternative models were compared to determine how best to describe variations in gape size with length. The first model assumed that a single linear relationship between length and gape height adequately described the relationship for all four eelpout species. The second model allowed for different intercepts, but assumed the same rate of increase for gape height with length

for the four eelpout species. The third model allowed for different intercepts and rates of increase for each eelpout species. The best model was chosen using the Akaike Information Criterion (AIC). All analyses were done in R version 3.0.3 (R Core Team 2016).

*Overall Diet Composition*

Multivariate tools were used to test for dissimilarities in diet composition among the four eelpout species and to investigate the influence of along-shelf (longitude, represented by transect, Fig. 1) and across-shelf (depth) spatial differences and total fish length on diet. Eastern (2013 and 2014) and central (2012) fishes were analyzed separately to avoid confounding effects of geographical dissimilarities and time. Diet information of eastern Beaufort Sea fishes was pooled and analyzed together if no interannual differences were detected. A Bray-Curtis dissimilarity matrix based on %W in individual stomachs was used in a PERMANOVA, with significance level set at $\alpha = 0.05$. Fish species, depth, and transect were included as fixed factors in the model, and fish length was included as a covariate. Due to the bathymetry over the sampling region, changes in latitude were closely associated with changes in depth. Therefore, latitude was excluded from the analysis to avoid issues with multicollinearity. Non-metric multidimensional scaling (nMDS) plot, based on the same Bray-Curtis dissimilarity matrix were created to show the similarities among individual stomach samples. All nMDS plots used Kruskal fit scheme 1 and were considered adequate when they had a maximum stress $\leq 0.2$. A similarity percentage (SIMPER) analysis was used to determine what percentage a given prey species contributed to the similarity (within groups) or dissimilarity (between groups) in diet composition of each *Lycodes* species. The prey items that contributed at least 70% of the cumulative observed similarities or dissimilarities in diet were reported. PERMANOVA, nMDS, and SIMPER analyses were conducted in PRIMER v.7.

Canonical correspondence analysis (CCA) was used to directly relate environmental (depth, along shelf (i.e., longitude), bottom temperature, and bottom salinity) and biological (fish total length) factors to diet composition. Due to smaller sample sizes in 2012 and less available corresponding environmental data, only pooled 2013 and 2014 data were used for CCA. In this analysis, prey composition (%W) of the coarse prey groups in each stomach was used as the multivariate response variable. All environmental variables were normalized to mean zero and standard deviation one prior to analysis. A permutation test of the CCA axes at a 5% significance level was used to test the null hypothesis that there is no overall association between the biotic (i.e., stomach contents) and environmental (i.e., fish length and environmental data) matrices. If the overall test was significant, permutation tests were used to assess the

significance of each individual term in the model, as well as the significance of each (constrained) CCA axis. In addition, results were examined graphically using a CCA plot, in which the length of a vector for a continuous factor indicates the magnitude of its effect on diet composition, and its direction in relation to a canonical axis indicates how much of the variability of the axis was explained by the given factor. The location of the weighted averages of each coarse prey group in the CCA plot in relation to these vectors was indicative of a variable's association with a given factor (Ter Braak 1986). All CCA analyses were conducted using the vegan 2.2-1 package in R, version 3.0.3.

*Size Class Analysis*

A combination of nMDS plots and clustering based on Bray-Curtis dissimilarity was used to determine size classes and ontogenetic shift in diet for three of the four eelpout species *L. adolfi, L. sagittarius*, and *L. seminudus*. The sample size for *L. polaris* was too small to use in this analysis. For each of the remaining eelpout species, fish were grouped into 10 mm size bins (e.g., 11 – 20, 21–30 mm size bin). A minimum of four fish was required for each size bin. If fewer than four fish were available for a 10-mm bin, consecutive size bins were combined with the next larger consecutive bin. Similarities among stomach samples grouped by fish size and described by %MW for each size group were visually examined using ordination plots. Cluster overlays were examined at varying resemblance levels (e.g., 40%, 50%, and 60% similarity) until ecologically reasonable groupings appeared.

*Stable Isotope Analysis*

Muscle tissue samples were collected for nitrogen and carbon stable isotope analyses from all individuals of the four species of *Lycodes* collected in 2014 (Table 2). Muscle clips were taken from above the lateral line towards the anterior end of each fish. A 5 x 5 mm section of muscle tissue was removed from each fish, making sure to exclude skin or bone as these tissues can have different isotopic signatures (Tieszen et al. 1983). Tissue samples were then placed in 0.5 ml microcentrifuge tubes and stored frozen until further processing. Subsamples of ten individual fish were selected within a 10-mm length bin for each eelpout species (e.g., 10–19 mm, 20–29 mm, 30–39 mm) to ensure sampling across the entirety of each species length range. Tissue samples for nitrogen stable isotope analysis were freeze-dried, crushed, and weighed. Tissue samples for carbon isotope analysis required additional processing. High lipid content in some fish muscle tissue impacts stable carbon isotope values, so lipid extraction (LE) was used to circumvent this issue (Pinnegar and Polunin 1999). Lipids were removed with a 2:1 chloroform: methanol solution. LE samples were allowed to dry overnight before being crushed and weighed. LE processing

can potentially impact stable nitrogen isotope signatures (Pinnegar and Polunin 1999), so samples to be measured for stable nitrogen isotope ratios were not lipid extracted. If an individual fish had insufficient tissues for both LE and non-LE analysis, only nitrogen values were determined. Isotope ratios were measured using Elemental Analysis-Isotope Ratio Mass Spectrometry (EA-IRMS) at the Alaska Stable Isotope Facility, using a Costech Elemental Analyzer (ECS 4010) and ThermoScientific Conflo IV interfaced with a ThermoScientific DeltaV Mass Spectrometer.

Table 2. Number of fish tissue samples for stable isotope analysis by species. Samples were collected in 2014. Two tissue samples were collected from each fish when possible, one each for nitrogen ($\delta^{15}N$) and carbon ($\delta^{35}C$).

| **Species** | **Samples for $\delta^{15}N$** | **Samples for $\delta^{13}C$** |
|---|---|---|
| *Lycodes adolfi* | 85 | 84 |
| *Lycodes polaris* | 16 | 10 |
| *Lycodes sagittarius* | 60 | 58 |
| *Lycodes seminudus* | 37 | 36 |
| **TOTAL** | **198** | **188** |

Stable isotope values were reported in standard delta notation ($\delta^{15}N$ and $\delta^{13}C$). Values were calculated with the equation:

$$\delta X = [(R_{sample} / R_{standard}) - 1] \times 1000$$

where X is $^{15}N$ or $^{13}C$ of a sample, and R is the corresponding isotopic ratio ($^{15}N/^{14}N$ or $^{13}C/^{12}C$). Standards used were atmospheric $N_2$ (atm) for nitrogen and Vienna Pee Dee Belemnite (VPDB) for carbon. Peptone was used as a laboratory standard and was analyzed every ten samples. A standard bi-plot was used to visualize differences in mean nitrogen and carbon isotope values among the four eelpout species. Stable isotope values were also plotted against total fish length to determine presence of ontogenetic shifts in eelpout TL ($\delta^{15}N$) and carbon source ($\delta^{13}C$).

Nitrogen and carbon stable isotope ratios from fish tissue were used to estimate TL and carbon sources, respectively, and to analyze ontogenetic changes in diet. Trophic level (TL) was calculated using the following equation:

$$TL_{fish} = (\delta^{15}N_{fish} - \delta^{15}N_{primary\ consumer})/3.4 + 2$$

Where $\delta^{15}N_{fish}$ is the stable nitrogen isotope signature for an individual eelpout, $\delta^{15}N_{primary\ consumer}$ is the stable nitrogen isotope value for a primary consumer (site specific average), in this case the brittle star *Ophiocten sericeum* (Bell et al. 2016), and 3.4‰ is the assumed enrichment step between trophic levels.

The $\delta^{15}N$ values for individual brittle stars were averaged by habitat (shelf vs. slope, i.e., $\leq$ 100 m and $\geq$ 200 m) and transect to account for spatial variability in $\delta^{15}N$ with across- and along-shelf sampling, which was evident in previous studies (Divine et al. 2015). A primary consumer instead of an actual primary producer source was used as baseline to integrate over the high spatial and temporal variability of primary producers, as it compares better to the time-integrated values of the fish consumers (Vander Zanden and Rasmussen 1999). Ophiuroids were assumed to have a TL of 2 for TL calculations. A stepwise enrichment of 3-4‰ in $\delta^{15}N$ was expected between subsequent trophic levels, and the widely used enrichment step of 3.4‰ was used for TL calculations (Hobson et al. 2002). A one-way ANOVA was used to test for significant differences in average TL among the four eelpout species. Comparisons of trophic levels among eelpout species and with changes in eelpout lengths were conducted to test for ontogenetic changes in trophic levels. An analysis of covariance (ANCOVA) using both linear and quadratic models was used to test if the relationship between length and $\delta^{15}N$, $\delta^{13}C$, or TL was different among the four eelpout species.

## Results

*Eelpout Species Confirmation*

The percentage of individuals with species identification agreement between morphometric methods and genomic/taxonomic ranged from 78% (*L. polaris*) to 85% (*L. seminudus*) (Table 3). *Lycodes adolfi* exhibited the second highest percent agreement between morphometric and genomic methods (84% overall percent agreement). The 15 fishes initially misidentified as *L. adolfi* were reassigned based on mtDNA as *L. sagittarius* (n=7), *L. seminudus* (n=7), and by the taxonomist as *L. squalmiventer* (n=1). The lowest percent agreement between morphometric and genomic methods was observed for *L. polaris* (78% overall). Fishes originally identified as *L. polaris* were re-identified based on mtDNA as *L. sagittarius* (n=1), *L. seminudus* (n=3), *L. eudipleurostictus* (n=1), *L. reticulatus* (n=1), and Shulupaoluk (*L. jugoricus*) (n=2) by the taxonomist. The six *L. seminudus* initially incorrectly identified were reassigned based on mtDNA as *L. adolfi* (n=1), Doubleline Eelpout (*L. euidipleurostictus*) (n=1), Arctic Eelpout (*L. reticulatus*) (n=2), Scalebelly Eelpout (*L. squalmiventer*) by taxonomist (n=1), and one was left at the genus level by the taxonomist because morphometric identification confirmation was not possible. *Lycodes sagittarius* exhibited the second lowest identification agreement of the four target *Lycodes* species (83% overall). Fishes originally identified as *L. sagittarius* by FOL were re-identified based on mtDNA as *L. adolfi* (n=1), *L. seminudus* (n=6), *L. euidipleurostictus* (n=1), and by the taxonomist as *L. squalmiventer* (n=1).

Of the 204 specimens included in the mtDNA analysis, 189 were conclusively identified as a single species; 15 individuals examined could not be unambiguously assigned to a single species. Sequences from those 15 samples yielded perfect or nearly perfect matches to those from more than one species represented in BOLD when the search was performed. For example, seven fish were identified as both *L. adolfi* and Pale Eelpout (*L. pallidus*) (n=4 for 2012, and n=3 for 2013). Sequences from all seven fish were most similar (> 99%) to barcode sequences of fish identified as *L. adolfi* in the BOLD database, but individuals identified as *L. pallidus* were also included in the BOLD list of potential matches. The cluster of BOLD sequence records that includes *L. adolfi* and *L. pallidus* includes variants as divergent as 1.9%. Sequences of seven eelpouts assigned as *L. seminudus* based on sequence match to BOLD archived specimens also closely matched with either Estuarine Eelpout (*L. tuneri*) and Saddled Eelpout (*L. mucosus*), but the degree of matching did not allow for a conclusive match to either species (n=4 for 2012 and n=3 for 2-13). Lastly, the cytochrome c oxidase 1 gene (COI) sequence from a specimen assigned as *L. polaris* was not distinguishable from *L. knipowitschi* (no common name; a potential synonym for *L.*

*mucosus*), and *L. tanakae* (no common name), two putative species that mtDNA sequences could not differentiate from each other or from *L. polaris*. In addition to the 15 fish discussed above, all fish (n=67) identified as *L. sagittarius* by mtDNA analysis were also matched to individuals identified as *L. marisalbi* in BOLD (these two species are not differentiated at the barcode DNA sequence). The inability to unambiguously match some barcode sequences to one recognized *Lycodes* species, and the low percent variation in base pairs across multiple species of eelpout indicates that there is significant genetic overlap between some currently recognized species of *Lycodes*, and suggests the presence of poorly differentiated species lineages in *Lycodes* where mtDNA lacks the level of genetic resolution to identify taxonomic or population boundaries. Based on the objectives of this project on biology and distribution, each individual fish that was identified as belonging to a species complex was treated as either *L. adolfi* (for the *L. adolfi* and *L. pallidus* complex), *L. polaris* (for the *L. polaris*, *L. knipowitschi*, and *L. tanakae* species complex), *L. sagittarius* (for the *L. sagittarius* and *L. marisalbi* complex), or *L. seminudus* (for the *L. seminudus, L. turneri,* and *L. mucosus* complex) based on currently available information on the genetic variability from BOLD and distinctiveness of these groups.

Table 3. Species confirmation results for four *Lycodes* species. The numbers of fish for mtDNA analysis are only those whose DNA was successfully isolated, amplified, and sequenced. The number identified by the University of Alaska Fisheries Oceanography Lab (FOL) or a taxonomist, the number of fish whose identity was confirmed as that identity assigned by FOL or a taxonomist, and the percent accuracy (% Accuracy) are given. Some individual fish could not be conclusively matched with only one known species by the Barcode of Life Database (BOLD), and instead were assigned to a species complex. No individual fishes were confirmed by both DNA and a taxonomist.

| | *L. adolfi*[1] | *L. polaris*[2] | *L. sagittarius*[3] | *L. seminudus*[4] |
|---|---|---|---|---|
| **Confirmed by mtDNA** | | | | |
| Total ID by FOL | 90 | 19 | 63 | 32 |
| ID Confirmed by mtDNA | 76 | 13 | 52 | 28 |
| % Accuracy | 84% | 68% | 83% | 88% |
| **Confirmed by Taxonomist** | | | | |
| Total ID by FOL | 6 | 17 | 9 | 8 |
| ID Confirmed by Taxonomist | 5 | 15 | 8 | 6 |
| % Accuracy | 83% | 88% | 89% | 75% |
| **Total Confirmed by mtDNA and Taxonomist** | | | | |
| Total ID by FOL | 96 | 36 | 72 | 40 |
| ID Confirmed | 81 | 28 | 60 | 34 |
| %Accuracy | 84% | 78% | 83% | 85% |

[1] *L. adolfi* includes fishes identified as *L. adolfi*, and fishes identified as part of the *L. adolfi/pallidus/esmarkii* species complex.
[2] *L. polaris* includes fishes identified as *L. polaris* and fishes identified as *L. polaris/tanakae/knipowitchi*.
[3] *L. sagittarius* includes fishes identified as *L. sagittarius* and as *L. sagittarius/marisalbi*.
[4] *L. seminudus* includes fishes identified as *L. seminudus* and fishes identified as *L. seminudus/mucosus/turneri*.

*Distribution and Length*

Eelpout distribution by species differed with depth but not longitude. The majority (94%) of all *L. polaris* by CPUE were collected at stations < 350 m depth (Figure 2). In 2012, five *L. polaris* were observed at 500 m (Figure 3). The other three eelpout species were collected mainly at depths ≥ 350 m; four *L. seminudus* collected between 10 and 100 m, and one *L. sagittarius* at 35 m were the exceptions (Figure 3). With respect to longitudinal distribution, all species except *L. adolfi* were found at all transects sampled.

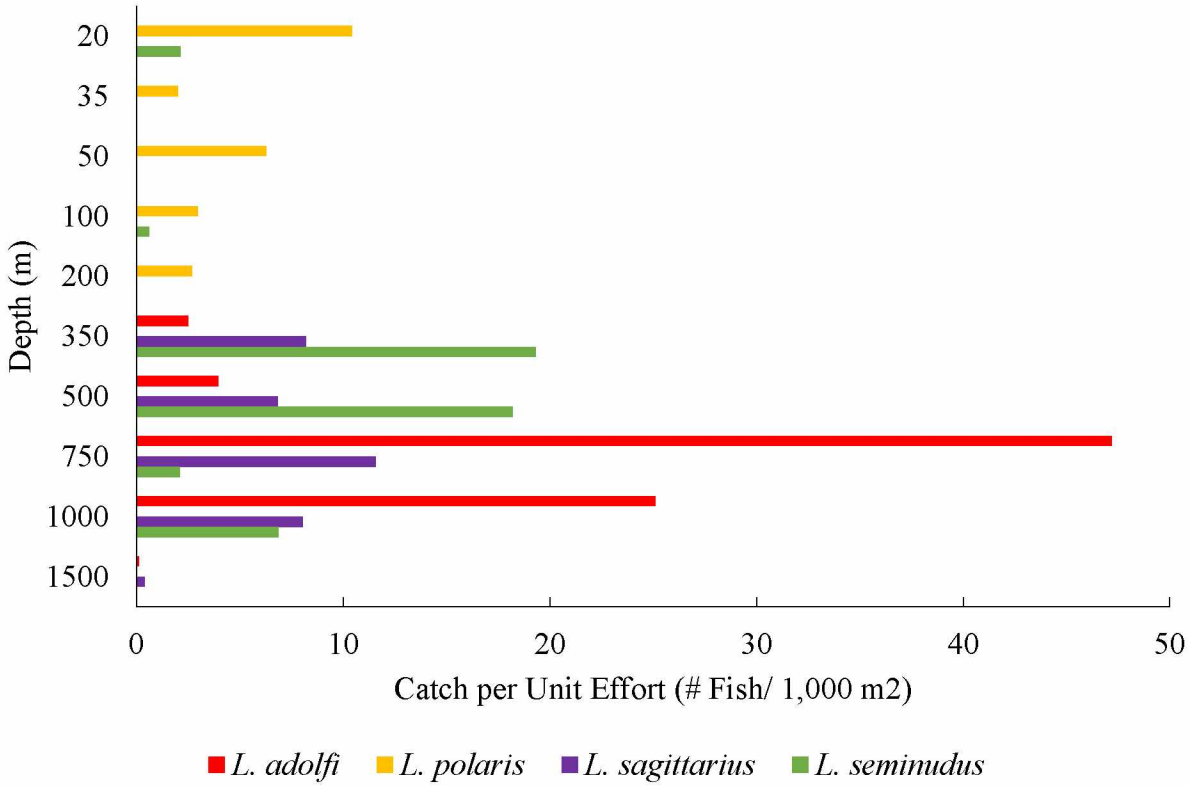


Figure 2. Catch per unit effort (CPUE) of *Lycodes* spp. collected at each sampling depth in 2013 and 2014. CPUE is presented as number of fish per 1,000 $m^2$ at each depth. Central (2012) Beaufort Sea trawls were non-quantitative, and are excluded from CPUE calculations.

All four eelpout species differed in body size ranges. *Lycodes sagittarius* had the largest observed individual at 472 mm total length (observed range: 56-472 mm; average 76 ± standard deviation of 33 mm). *Lycodes seminudus* had the second largest individual observed at 465 mm (52-465 mm; ± 111 mm). *Lycodes polaris* (42–205 mm; 76 ± 33 mm) had the second smallest maximum total length observed and *L. adolfi* (38–182 mm; 103 ± 39 mm) had the smallest. The largest fishes were collected at depths ≥ 350 m (Figure 3). A Kruskal-Wallis one-way ANOVA based on ranks indicated differences in mean length among the four eelpout species ($H = 199.668$, $p = < 0.001$). Subsequent paired tests based on Dunn's method indicated all combinations of the four eelpout species were significantly different from each other ($p < 0.05$).

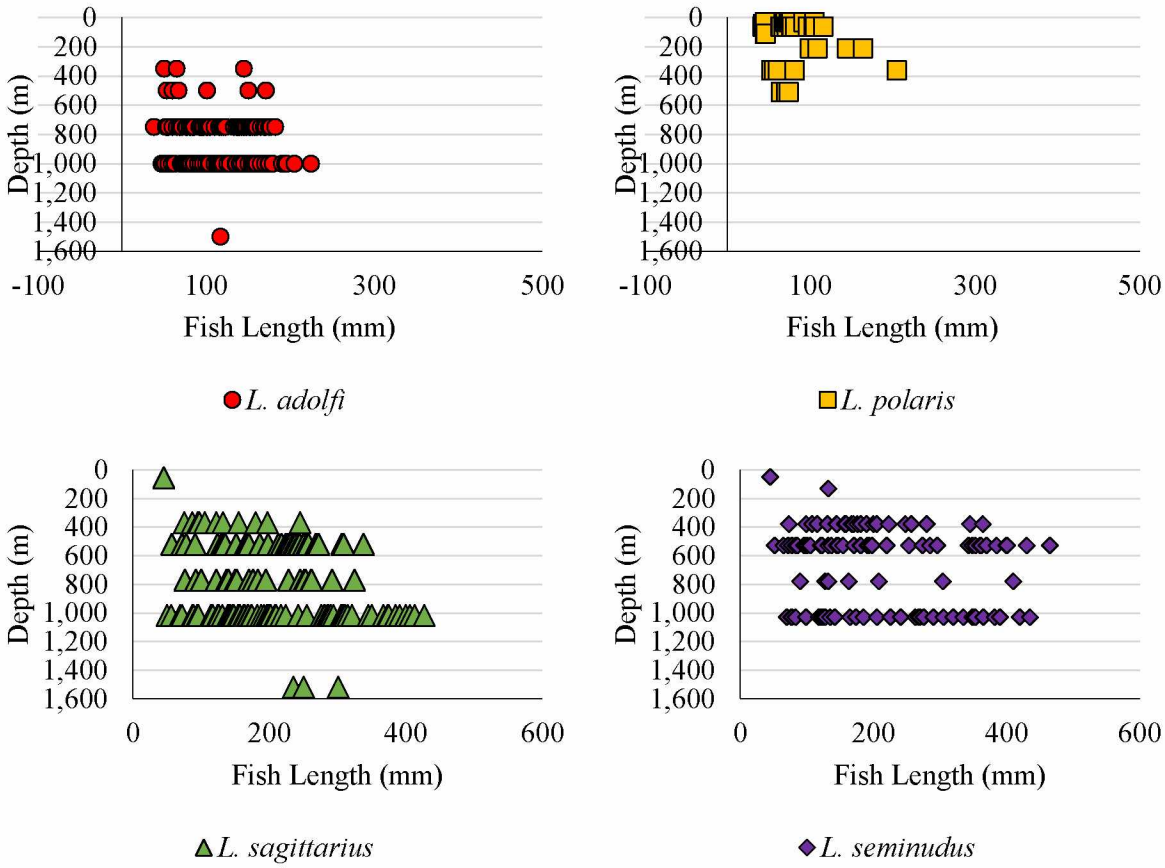


Figure 3. Total fish length at depth for all of the four eelpout species collected in the central (2012) and eastern (2013/2014) Beaufort Sea. Each point represents an individual fish at a specific depth.

*Gape Size*

Gape size increased linearly with total fish length for all four eelpout species, but the best model (smallest AIC) indicated that the rate of increase differed among species (Figure 4). Maximum gape height was largest for *L. seminudus* (67 mm) and smallest for *L. polaris* (12 mm) (Table 4). *Lycodes sagittarius* and *L. adolfi* had maximum gape heights of 20 and 38 mm, respectively. *Lycodes seminudus* had the largest gape height at a given length, followed by *L. adolfi, L. polaris* and lastly *L. sagittarius* (Figure 4). Coefficients of determination of the linear relationship ranged from $R^2 = 0.68$ for *L. polaris* to $R^2 = 0.89$ for *L. sagittarius*. The slope was lowest for *L. sagittarius* (0.09), and similar for all other eelpouts (0.1 for *L. adolfi* and *L. seminudus*, 0.104 for *L. polaris*). It is important to note that *L. adolfi* and *L. polaris* were, on average, smaller than *L. seminudus* and *L. sagittarius*, and they did not reach similar maximum lengths observed for the other two eelpout species. Length of prey consumed increased with increase in gape height at length, though outliers were present for all four *Lycodes* species (Figure 4).

Table 4. Gape height (mm) measurements and relation to total fish length (mm) for four eelpout species collected in 2014. Sample size (n), maximum (Max), minimum (Min), average, and standard deviation (StdDev) for gape height for each eelpout species. Results of comparison of analysis of covariance (ANCOVA) for the best model according to AIC are given.

| Gape Height Summary | | | | | |
|---|---|---|---|---|---|
| Species | n | Max | Min | Average | StdDev |
| *Lycodes adolfi* | 74 | 19.7 | 4.3 | 11.4 | 4.3 |
| *Lycodes polaris* | 18 | 11.7 | 3.0 | 5.5 | 2.4 |
| *Lycodes sagittarius* | 55 | 37.6 | 5.2 | 15.5 | 7.4 |
| *Lycodes seminudus* | 37 | 67.2 | 4.1 | 25.6 | 15.5 |
| Grand Total | 184 | | | | |

| Comparison of ANCOVA Models - All Four Eelpout Species | | |
|---|---|---|
| | AIC | Model |
| Model 1 | 1,084.9 | Gape Height = b * Length |
| Model 2 | 1,022.5 | Gape Height = Species_k + b*Length |
| **Model 3** | **1,014.9** | **Gape Height = Species_k + b_k*Length** |

| ANCOVA for Model 3 | | | | | |
|---|---|---|---|---|---|
| | DF | SumSq | MeanSq | F value | Pr(>F) |
| Species | 3 | 5,874.5 | 1,958.2 | 138.5 | < 2.2e-16 |
| Length | 1 | 11,268.3 | 11,268.3 | 797.2 | < 2.2e-16 |
| Species:Length | 3 | 199.9 | 66.6 | 4.7 | 0.003436 |
| Residuals | 175 | 2,473.5 | 14.1 | | |

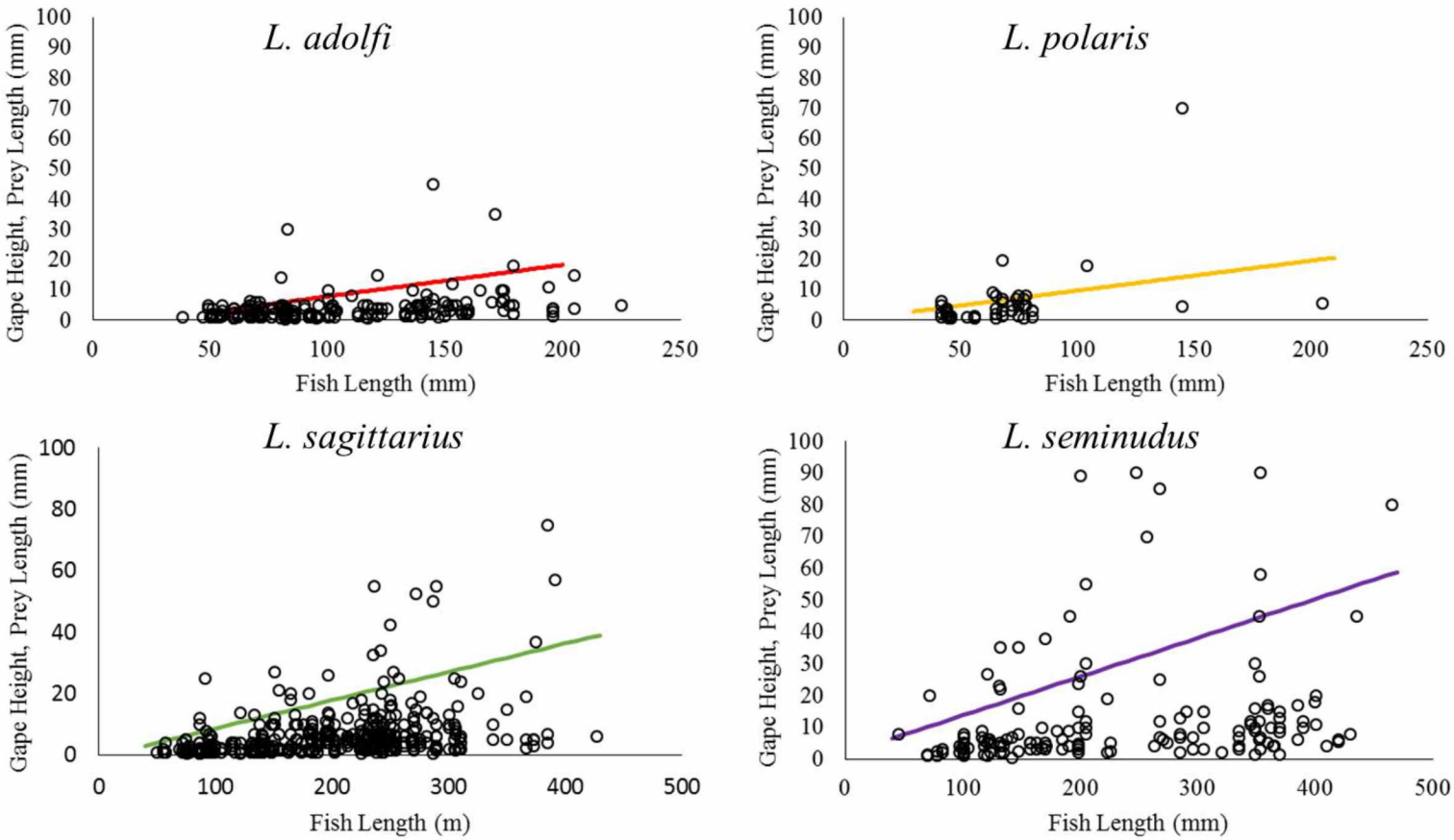


Figure 4. Mean estimated gape height (lines, mm) and individual prey lengths (circles, mm) at a given length for each eelpout species. Gape height was estimated by linear regression on length with species-specific slopes.

*Stomachs Processed*

Out of 466 available stomachs for the four eelpout species, 97 (21% of total available stomachs) were excluded. Excluded stomachs were empty, burst or contained only parasites (Table 1). Parasitic nematodes were the only contents of 13 (3% of total available) of the stomachs; these resident parasites were not actively consumed, so the stomachs were excluded from the analysis. The proportion of non-empty stomachs was highest for *L. sagittarius* (86%), and similar among *L. adolfi* (75%), *L. polaris* (77%), and *L. seminudus* (77%). The percent of non-empty stomachs differed with changes in depth and fish length (Figure 5). There were more empty stomachs at depths of 350, 500, 750, and 1,000 m (n = 81, 17% of total available stomachs) than at shallower depths (n = 9 at ≤ 200 m, 1.9% of total available stomachs). However, the proportion of empty stomachs was highest at the two shallowest depths (20 m and 35 m). Average fish length increased with increasing depth.

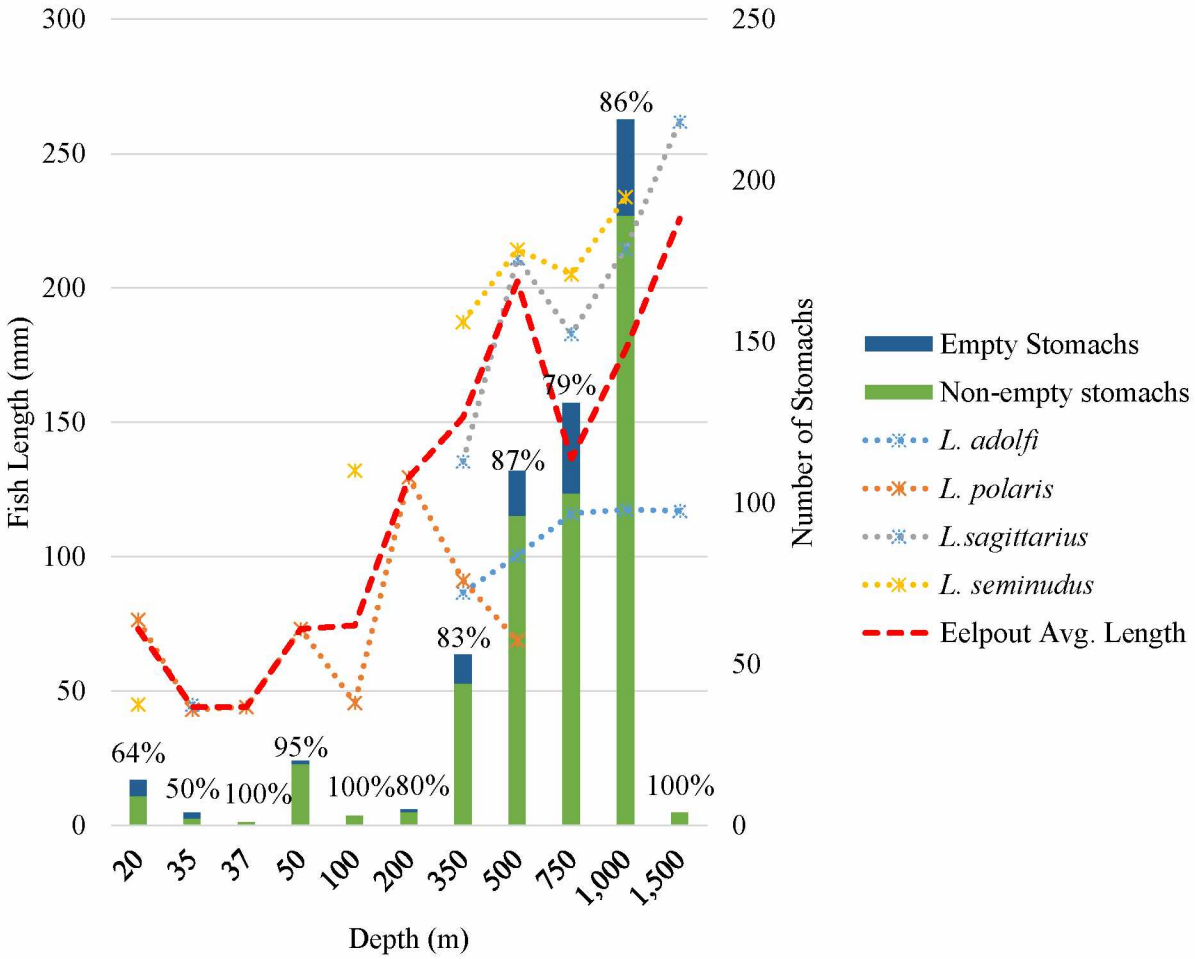


Figure 5. Number of empty (blue bars) and non-empty (green) stomachs at each collection depth and average length by species and depth (lines). The percent of non-empty stomachs at collection depth is given above each bar. Average length (mm) of all four eelpout species at depth is given by the red dashed line.

*Prey Groups*

In total, 106 distinct fine resolution prey types were observed, representing 14 coarse prey groups (Table 5 and 6). The greatest number of distinct fine resolution prey items (n = 15 in 2012 and n = 19 in 2013/2014) were types of polychaetes. The next most diverse group was Amphipoda (n = 8 in 2012 and n = 11 in 2013/2014). The majority of amphipods that could be identified to family, genus, or species level were benthic (98%). The exception was the pelagic genus *Themisto* (n=1 in 2013/2014). Other pelagic amphipod species may be represented in the unidentified amphipod group (Amphipoda Unid.). Teleost prey were found in *L. seminudus* stomachs. Of the five fish prey observed, four were identified as Arctic Cod (*Boreogadus saida*) and one could not be identified. Length of prey ranged from very small Foraminifera (average 1.4 ± 0.6 SD mm) and Ostracoda (average 1.0 ± 0.7 SD mm) to large Polychaeta (average 16 ± 15.3 SD mm) and fish (90.0 mm for the one individual measured) (Table 7 and Figure 6).

The average number of fine resolution prey items per fish stomach differed among eelpout species in the central (Kruskal-Wallis one-way ANOVA: H = 33.173, P = <0.001) and eastern (Kruskal-Wallis one-way ANOVA: H = 42.997, P = <0.001) Beaufort Sea. On average, *L. sagittarius* had the largest number of fine resolution prey items per stomach (4.0 in 2012 and 8.7 prey per individual fish in 2013/2014). The lowest average number of prey per stomach was observed in *L. adolfi* (1.1 in 2012) and *L. seminudus* (2.5 in 2013/2014). The number of prey items per stomach for *L. sagittarius* in the central Beaufort Sea was significantly higher than *L. adolfi* (Dunn's method: Diff. of Ranks = 61.9, Q = 5.478), but not *L. polaris* (Diff. of Ranks = 27.2, Q = 2.575), and *L. seminudus* could not be tested due to unequal sample size. In the eastern Beaufort Sea, the number of prey items per stomach for *L. sagittarius* differed from *L. seminudus* (Diff. of Ranks = 75.1, Q = 5.151), *L. adolfi* (Diff. or Ranks = 74.7, Q = 6.054), and *L. polaris* (Diff. of Ranks = 71.8, Q = 2.866). The number of items in each stomach of *L. polaris* in the central Beaufort Sea did not differ significantly from *L. adolfi* (Diff. of Ranks = 34.6, Q = 2.617) and could not be tested for *L. seminudus*. In the eastern Beaufort Sea, *L. polaris* did not differ from *L. seminudus* (Diff. of Ranks = 3.3, Q = 0.123) and could not be tested against *L. adolfi*.

Prey types were then grouped into 14 coarse taxonomic groups for all subsequent analyses. Cumulative prey curves for the eastern Beaufort Sea illustrated the need for aggregating prey at a coarser taxonomic level (Figure 7). Similar results were seen for the central Beaufort Sea.

Table 5. Prey groups found in eelpout stomachs collected in the central Beaufort Sea (2012). Coarse prey groups are presented in phylogenetic order and indicated in boldface. Prey contributing to each coarse prey group are listed below. Numbers of prey collected from stomachs of each eelpout species are also presented, where n is the number of stomachs of each *Lycodes* spp. examined, excluding those that were empty or contained only parasites.

| Prey Group | *L. adolfi* (n=25) | *L. polaris* (n=30) | *L. sagittarius* (n=75) | *L. seminudus* (n=39) |
|---|---|---|---|---|
| **Foraminifera** | | | **1** | |
| **Mollusca** | **1** | | **15** | **4** |
| Bivalvia Unid. | | | 3 | |
| *Ennucula tenuis* | | | 1 | 1 |
| *Thyasira flexuosa* | | | 1 | |
| Thyasiridae | | | 5 | 2 |
| Yoldiidae | 1 | | 5 | 1 |
| **Polychaeta** | **3** | **10** | **89** | **22** |
| *Cossura* spp. | | | 1 | |
| *Harmothoe* spp. | | 1 | | |
| *Levinsenia gracilis* | | | 1 | |
| Lumbrineridae | | | 3 | 1 |
| *Lumbrineris* spp. | | | 1 | |
| *Maldane sarsi* | | | 1 | |
| Maldanidae | | | 1 | |
| Nephtyidae | | | 3 | 1 |
| *Nephtys* spp. | | 1 | 1 | |
| Opheliidae | | | 1 | 2 |
| *Ophelina* spp. | | | 4 | |
| Paraonidae | | | 1 | |
| Polychaeta Unid. | 2 | 8 | 52 | 10 |
| Polynoidae | 1 | | 3 | 2 |
| Spionidae | | | 16 | 6 |
| **Copepoda** | **5** | **5** | **17** | **5** |
| Aetideidae | 1 | | 1 | |
| *Calanus glacialis* | | | 1 | |
| Copepoda Unid. | | 4 | 1 | |
| Harpacticoida | 4 | 1 | 11 | 5 |
| *Metridia longa* | | | 3 | |

| Prey Group | *L. adolfi* (n=25) | *L. polaris* (n=30) | *L. sagittarius* (n=75) | *L. seminudus* (n=39) |
|---|---|---|---|---|
| **Ostracoda** | | **2** | | **1** |
| **Cumacea** | **1** | **10** | **18** | |
| *Campylopus* spp. | | 1 | | |
| Cumacea Unid. | 1 | 4 | 2 | |
| *Diastylis* spp. | | 1 | | |
| *Ektonodiastylis robusta* | | | 1 | |
| *Eudorella emarginata* | | | 9 | |
| *Eudorella* spp. | | | 1 | |
| *Leucon* spp. | | 4 | 3 | |
| Leuconidae | | | 2 | |
| **Tanaidacea** | | **1** | **3** | **1** |
| **Isopoda** | **4** | **1** | **38** | **15** |
| Gnathiidae | | | | 1 |
| Idoteidae | | | 1 | |
| Isopoda Unid. | 4 | 1 | 37 | 14 |
| **Amphipoda** | **4** | **16** | **69** | **11** |
| *Aceroides latipes* | 2 | | 35 | 7 |
| *Aceroides* spp. | | | 3 | |
| Amphipoda Unid. | 1 | 11 | 17 | 3 |
| *Anonyx* spp. | | | 2 | |
| Gammaridea | | | 1 | |
| Lysianassidae | 1 | | 8 | |
| Oedicerotidae | | 5 | 3 | |
| *Rhachotropis* spp. | | | | 1 |
| **Crustacea Unid.** | **8** | **14** | **27** | **12** |
| **Ophiuroidea** | | **1** | | **2** |
| *Ophiura sarsii* | | 1 | | |
| Ophiuroidea | | | | 2 |

Table 5. Continued from previous page.

| Prey Group | *L. adolfi* (n=25) | *L. polaris* (n=30) | *L. sagittarius* (n=75) | *L. seminudus* (n=39) |
|---|---|---|---|---|
| **Teleost** | | | **1** | **1** |
| Teleost Unid. | | | 1 | 1 |
| **Animal Unid.** | | **2** | **3** | |
| **Other** | 1 | 1 | 3 | 3 |
| Caridea | | | 1 | 1 |
| Decapoda Unid. | | | 1 | |
| Diptera | | 1 | | |

| Prey Group | *L. adolfi* (n=25) | *L. polaris* (n=30) | *L. sagittarius* (n=75) | *L. seminudus* (n=39) |
|---|---|---|---|---|
| **Other - continued** | **1** | **1** | **3** | **3** |
| Mysidacea Unid. | | | | 2 |
| Nemertea | | | 1 | |
| Paguridae | 1 | | | |
| **Total** | **27** | **67** | **302** | **91** |
| **Avg.** | **1.1** | **2.2** | **4.0** | **2.3** |

Table 6. Prey groups found in eelpout stomachs collected in the eastern Beaufort Sea (2013/2014). Coarse prey groups are presented in phylogenetic order and indicated in boldface. Prey contributing to each coarse prey group are listed below. Numbers of prey collected from stomachs of each eelpout species are also presented, where n is the number of stomachs of each *Lycodes* spp. examined, excluding those that were empty of contained only parasites.

| Prey Group | *L. adolfi* (n=139) | *L. polaris* (n=13) | *L. sagittarius* (n=76) | *L. seminudus* (n=68) |
|---|---|---|---|---|
| **Foraminifera** | **24** | | **19** | |
| **Mollusca** | **7** | | **33** | **1** |
| Bivalvia | 6 | | 22 | |
| Gastropoda | | | 1 | |
| Mollusca Frag. | | | 1 | |
| *Musculus* spp. | | | 1 | |
| *Nuculana* spp. | | | 1 | |
| Rhabdidae | | | 1 | |
| Scaphopoda | | | 3 | |
| Yoldiidae | 1 | | 3 | 1 |
| **Polychaetea** | **36** | **4** | **229** | **49** |
| Lumbrineridae | | | 1 | |
| *Maldane sarsi* | 2 | | 46 | 19 |
| Maldanidae | | | 2 | |
| Nephtyidae | 3 | | 7 | 3 |
| *Onuphis parva* | | | 4 | |
| *Onuphis* spp. | | | 4 | 1 |
| Opheliidae | 2 | | 51 | |
| *Ophelina* spp. | | | 5 | |
| Oweniidae | | | | 7 |
| *Paradiopatra parva* | | | | 1 |
| *Paradiopatra* spp. | | | 1 | |
| Paraonidae | | | 1 | |
| Phyllodocidae | | | | 1 |
| Polychaeta Unid. | 18 | 2 | 33 | 5 |
| Polychaeta Frag. | 8 | 1 | 16 | 5 |
| Polynoidae | 1 | | 4 | 5 |
| Polynoidae Frag. | 1 | 1 | | 1 |
| Sabellidae | | | 2 | |

| Prey Group | *L. adolfi* (n=139) | *L. polaris* (n=13) | *L. sagittarius* (n=76) | *L. seminudus* (n=68) |
|---|---|---|---|---|
| **Polychaetea - Continued** | | | | |
| Spionidae | 1 | | 25 | |
| Terebellidae | | | 24 | |
| *Terebellides* spp. | | | 2 | 1 |
| Trichobranchidae | | | 1 | |
| **Copepoda** | **124** | **14** | **100** | **3** |
| Aetideidae | 1 | | | |
| Calanoida | 10 | | | |
| *Calanus hyperboreus* | 1 | | | |
| Copepoda Unid. | 5 | | 2 | 1 |
| Cyclopoida | 2 | | 8 | |
| *Euchaeta* spp. | | | | 1 |
| Harpacticoida | 98 | 14 | 90 | |
| *Metridia longa* | 4 | | | 1 |
| *Metridia* spp. | 2 | | | |
| *Paraeuchaeta norvegica* | 1 | | | |
| **Ostracoda** | **37** | **3** | **28** | **1** |
| **Cumacea** | **9** | **3** | **9** | **1** |
| Cumacea Unid. | 6 | 3 | 4 | |
| *Diastylis* spp. | | | 2 | |
| *Ektonodiastylis robusta* | 1 | | 3 | 1 |
| *Eudorellopsis* spp. | 2 | | | |
| **Tanaidacea** | **32** | **1** | **43** | **4** |
| **Isopoda** | **48** | | **138** | **24** |
| Gnathiidae | 4 | | 4 | 2 |
| Isopoda Unid. | 42 | 0 | 134 | 19 |
| Isopoda Frag. | 1 | | | 1 |
| *Saduria entomon* | 1 | | | 1 |
| *Synidotea* spp. | | | | 1 |

Table 6. Continued from previous page.

| Prey Group | *L. adolfi* (n=139) | *L. polaris* (n=13) | *L. sagittarius* (n=76) | *L. seminudus* (n=68) |
|---|---|---|---|---|
| **Amphipoda** | **25** | **7** | **25** | **10** |
| *Aceroides latipes* | 7 | 1 | 4 | |
| *Aceroides* spp. | 4 | | 2 | 1 |
| Amphipoda Unid. | 8 | 4 | 6 | 8 |
| *Haploops* spp. | | | 2 | |
| *Hippomedon* spp. | 1 | | | |
| Lysianassidae | 5 | | 6 | |
| Oedicerotidae | | 2 | 1 | 1 |
| *Orchomene* spp. | | | 1 | |
| Phoxocephalidae | | | 1 | |
| *Rhachotropis* spp. | | | 1 | |
| *Themisto* spp. | | | 1 | |
| **Crustacea Unid.** | **41** | **3** | **18** | **11** |

| Prey Group | *L. adolfi* (n=139) | *L. polaris* (n=13) | *L. sagittarius* (n=76) | *L. seminudus* (n=68) |
|---|---|---|---|---|
| **Ophiuroidea** | | | **6** | **60** |
| Ophiuroidea | | | 6 | 55 |
| Ophiuroidea Frag. | | | | 5 |
| **Teleost** | | | | **4** |
| *Boreogadus saida* | | | | 4 |
| **Animal Unid.** | **5** | **1** | **2** | **2** |
| **Other** | **2** | | **9** | **1** |
| Euphausiacea | | | | 1 |
| Fish egg | | | 1 | |
| Mysidacea | 2 | | | |
| Sipuncula | | | 8 | |
| **TOTAL** | **390** | **36** | **659** | **171** |
| **Avg. TOTAL** | **2.8** | **2.8** | **8.7** | **2.5** |

Table 7. Length of coarse prey groups consumed. Length (mm) was measured for individuals in each prey group. Minimum (Min), maximum (Max), average (Avg), and standard deviation (StDev) of length of each prey group are presented.

| Prey Groups | n | Min Length (mm) | Max Length (mm) | Avg Length (mm) | StDev |
|---|---|---|---|---|---|
| Foraminifera | 26 | 1.0 | 3.0 | 1.4 | 0.6 |
| Mollusca | 35 | 1.0 | 13.0 | 3.0 | 3.1 |
| Polychaeta | 207 | 2.5 | 70.0 | 16.0 | 15.3 |
| Copepoda | 213 | 0.3 | 14.0 | 1.8 | 1.8 |
| Ostracoda | 64 | 0.5 | 3.0 | 1.0 | 0.7 |
| Cumacea | 40 | 2.0 | 9.0 | 4.9 | 1.8 |
| Tanaidacea | 66 | 1.0 | 7.0 | 3.6 | 1.6 |
| Isopoda | 165 | 1.0 | 45.0 | 5.6 | 9.0 |
| Amphipoda | 53 | 2.0 | 35.0 | 6.9 | 5.8 |
| Ophiuroidea | 44 | 3.0 | 6.0 | 4.5 | 0.9 |
| Teleost | 1 | 90.0 | 90.0 | 90.0 | - |
| Other | 11 | 4.0 | 42.5 | 17.9 | 15.8 |

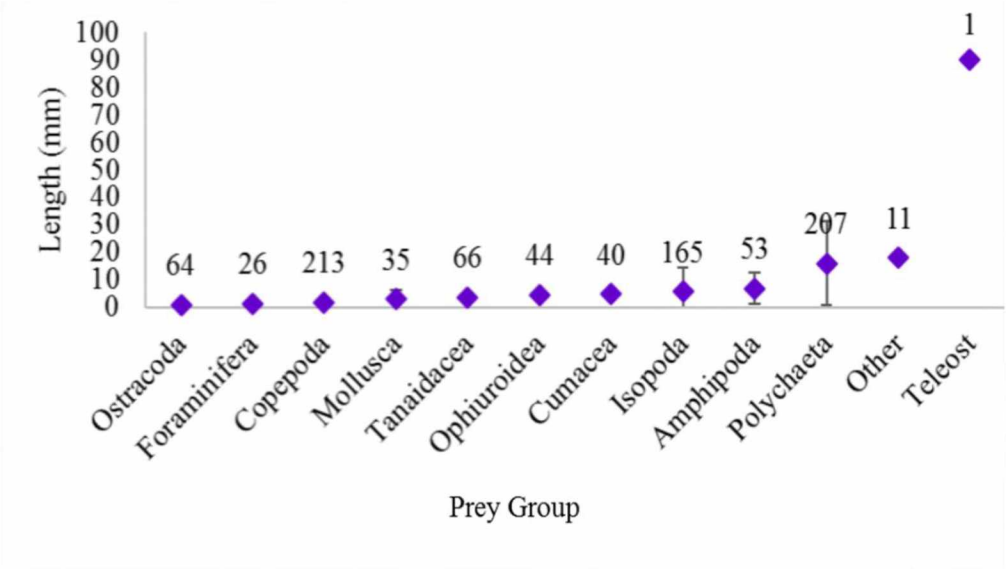


Figure 6. Average prey lengths of coarse prey groups consumed. Standard deviation is indicated by error bars. Number of individual prey represented by each group is displayed. Length was not measured for unidentified crustaceans (Crustacea Unid.) or unidentifiable animals (Animal Unid.).

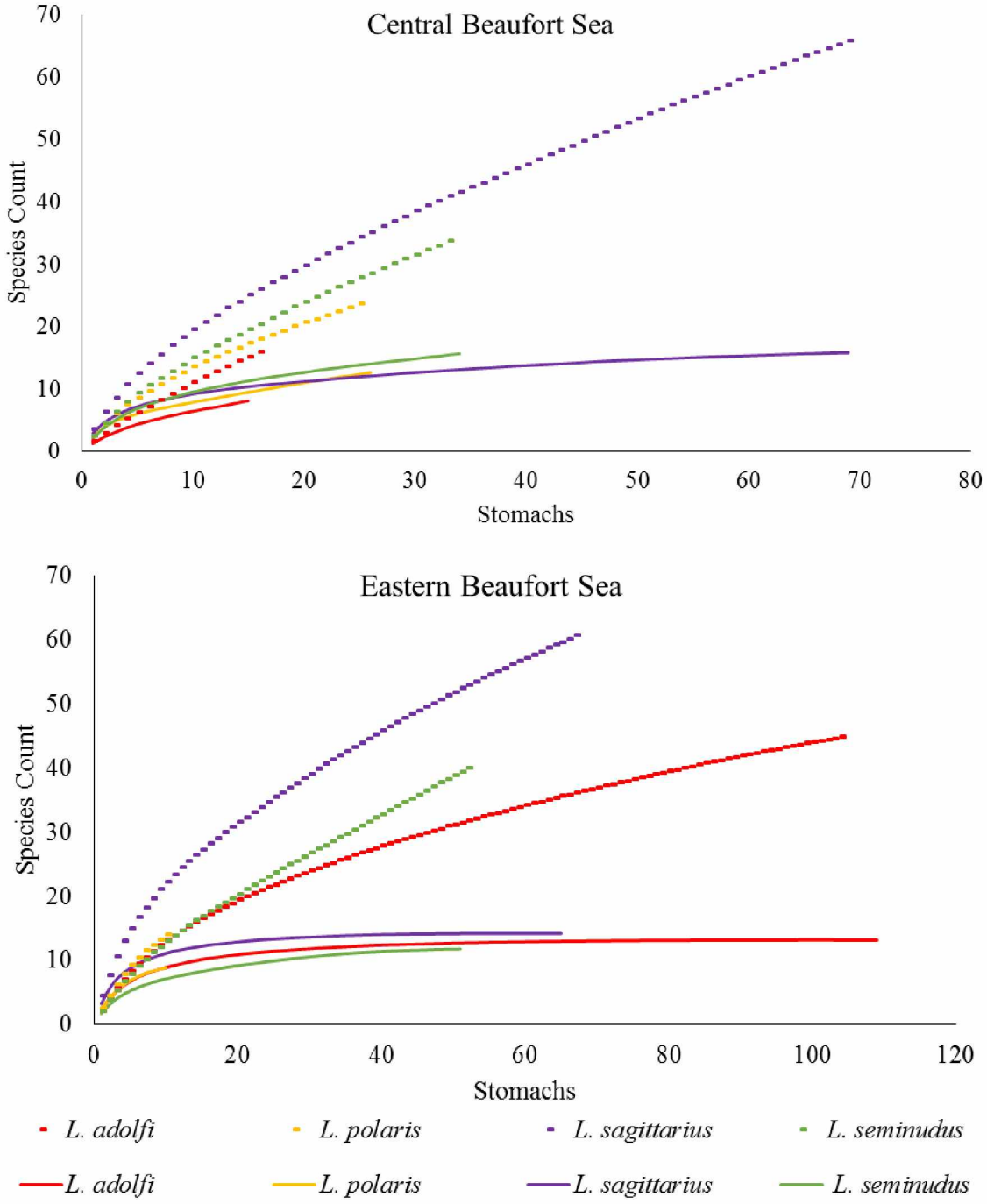


Figure 7. Cumulative prey curves for the four eelpout species collected in the central and eastern Beaufort Sea. Prey grouped at the lowest taxonomic level possible are indicated by dashed lines. Coarse grouped prey are represented by solid lines.

*Interannual Differences in Diet*

Samples collected were pooled across years when interannual differences were not significant. Interannual diet composition did not differ significantly in the eastern Beaufort Sea, between 2013 and 2014 (*L. adolfi*: PERMANOVA, $t = 0.98$, $p = 0.44$; *L. polaris*: no test; *L. sagittarius*: $t = 1.24$, $p = 0.14$; *L. seminudus*: $t = 0.88$, $p = 0.58$). The central Beaufort Sea was only sampled in 2012, so no interannual comparison was possible for this region. In the eastern Beaufort Sea only one *L. polaris* stomach was available from 2013, and, therefore, *L. polaris* was not included in the interannual analysis. Pooling of samples collected in the same region (central or eastern) was done if cumulative prey curves indicated pooling was necessary. Prey curves indicated that sample size in 2013 was too small to be adequately described for three of the four *Lycodes* species, but adequate for all species in 2014 except *L. polaris* (Figure 8). Cumulative prey curves of pooled eastern Beaufort Sea specimens indicated diet for all species except *L. polaris* was adequately described with the available sample sizes.

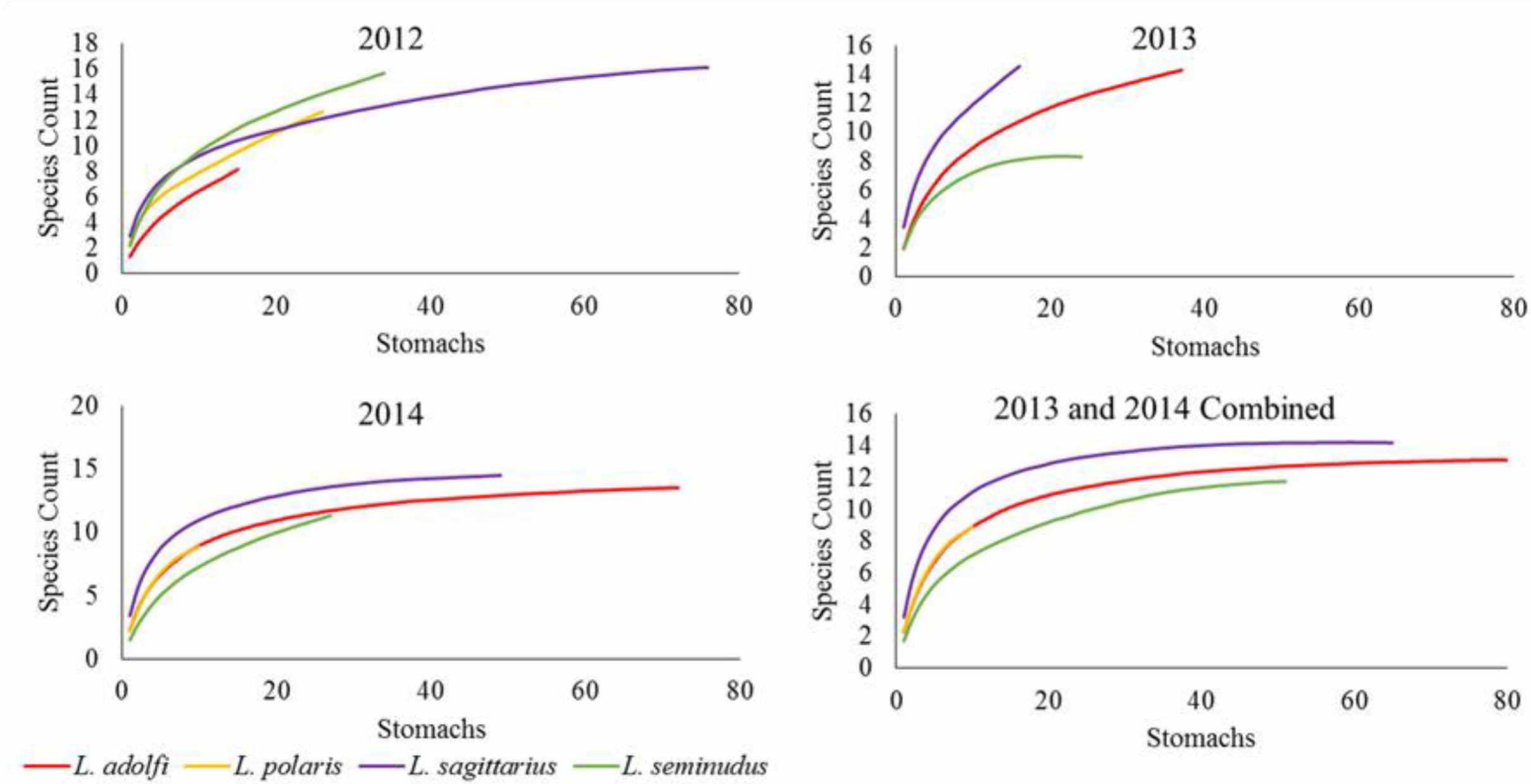


Figure 8. Cumulative prey curves for four *Lycodes* species using coarse prey groups. The sampling years 2012, 2013, 2014, and combined 2013 and 2014 are represented. Diet is adequately described at a sample size that corresponds to the cumulative prey curve reaching an asymptote. In 2013, only one *L. polaris* was available and was not included in this analysis.

*Overall Diet Composition*

Diet composition was significantly related to eelpout species, length, depth, and transect. Diets varied significantly with total length and with depth, while differences among transects were only significant in 2012 (Table 8) and not in 2013/2014 (Table 9). The interaction between fish length and fish species was significant for both 2012 and 2013/2014 fishes. An additional interaction between depth and transect was significant for 2012, as was the interaction between species and transect for 2013/2014. For 2012 samples, diet composition was significantly different between *L. polaris* and *L. adolfi* and between *L. polaris* and *L. sagittarius*. Pair-wise tests in 2013/2014 indicated that diet composition was different between all pairings except between *L. adolfi* and *L. polaris* and between *L. polaris* and *L. seminudus.* The scatter of points in the nMDS plots indicated a high level of intraspecific variability in diet compositions within each of the four eelpout species and considerable overlap among species (Figures 9 and 10).

Table 8. Permutational analysis of variance (PERMANOVA) results for diet composition among four eelpout species collected in the central Beaufort Sea in 2012 using percent weight (%W) of prey items. Species, depth, and transect were fixed factors. Length was a continuous covariate. Analysis used Type 1 sums of squares and permutation of residuals under a reduced model. Pair-wise tests were conducted for factors that were significant ($\alpha$=0.05). For pair-wise tests of depth and transect, all combinations were tested but only significant pairs are presented. Degrees of freedom (df), sums of squares (SS), mean squares (MS), pseudo F statistic (Pseudo-F), t statistic (t), P values (P(perm)), and the number of unique permutations (Perm) are given.

| Source | df | SS | MS | Pseudo-F | p(Perm) | Perm |
|---|---|---|---|---|---|---|
| **Length** | **1** | **19628** | **19628.0** | **6.6587** | **0.001** | **999** |
| **Species** | **3** | **21165** | **7055.1** | **2.3935** | **0.004** | **998** |
| **Depth** | **5** | **47354** | **9470.9** | **3.2130** | **0.001** | **998** |
| **Transect** | **2** | **18009** | **9004.5** | **3.0548** | **0.004** | **998** |
| **Length x Species** | **3** | **26429** | **8809.7** | **2.9887** | **0.002** | **996** |
| Length x Depth | 3 | 10105 | 3368.4 | 1.1427 | 0.334 | 999 |
| Length x Transect | 2 | 8491 | 4245.2 | 1.4402 | 0.155 | 999 |
| Species x Depth | 1 | 3120 | 3119.9 | 1.0584 | 0.357 | 998 |
| Species x Transect | 3 | 8392 | 2797.5 | 0.9490 | 0.506 | 999 |
| **Depth x Transect** | **2** | **18297** | **9148.3** | **3.1036** | **0.001** | **998** |
| Species x Depth x Transect | 1 | 1332 | 1331.6 | 0.4517 | 0.825 | 997 |
| Residuals | 115 | 3.39E+05 | 2948 | | | |
| Total | 141 | 5.21E+05 | | | | |

| Pair-Wise Test: Species | t | p(Perm) | Perm |
|---|---|---|---|
| ***Lycodes adolfi, Lycodes polaris*** | **1.6195** | **0.035** | **999** |
| *Lycodes adolfi, Lycodes sagittarius* | 1.1617 | 0.208 | 998 |
| *Lycodes adolfi, Lycodes seminudus* | 0.8363 | 0.679 | 998 |
| ***Lycodes polaris, Lycodes sagittarius*** | **1.7995** | **0.009** | **999** |
| *Lycodes polaris, Lycodes seminudus* | 1.2008 | 0.200 | 996 |
| *Lycodes sagittarius, Lycodes seminudus* | 1.4967 | 0.053 | 999 |

| Pair-Wise Test: Depth | t | p(Perm) | Perms |
|---|---|---|---|
| **500, 1000** | **3.1097** | **0.001** | **999** |

| Pair-Wise Test: Transect | t | P(perm) | Perms |
|---|---|---|---|
| **B1, B2** | **1.6345** | **0.023** | **998** |
| **B1, BX** | **1.8092** | **0.014** | **999** |

Table 9. Permutational analysis of variance (PERMANOVA) results for diet composition among four eelpout species collected in the eastern Beaufort Sea in 2013 and 2014 using percent weight (%W) of prey items. Species, depth, and transect were included as fixed factors. Length was included as a covariate. Analysis used Type 1 sums of squares and permutation of residuals under a reduced model. Pair-wise tests were conducted for significant factors ($\alpha$=0.05). For pair-wise test of depth all combinations were tested, but only significant pairs are presented. Degrees of freedom (df), sums of squares (SS), mean squares (MS), pseudo F statistic (Pseudo-F), t statistic (t), P values (P(perm)), and the number of unique permutations (Perm) are given.

| Source | df | SS | MS | Pseudo-F | p(Perm) | Perm |
|---|---|---|---|---|---|---|
| **Length** | **1** | **37801** | **37801** | **11.4670** | **0.001** | **999** |
| **Species** | **3** | **38774** | **12925** | **3.9206** | **0.001** | **999** |
| **Depth** | **9** | **77429** | **8603** | **2.6097** | **0.001** | **996** |
| Transect | 6 | 25795 | 4299 | 1.3041 | 0.110 | 998 |
| **Length x Species** | **3** | **30884** | **10295** | **3.1228** | **0.001** | **999** |
| Length x Depth | 8 | 24498 | 3062 | 0.9289 | 0.624 | 997 |
| Length x Transect | 6 | 17178 | 2863 | 0.8685 | 0.705 | 996 |
| **Species x Depth** | **7** | **30741** | **4392** | **1.3321** | **0.071** | **997** |
| **Species x Transect** | **13** | **61070** | **4698** | **1.4250** | **0.009** | **997** |
| Depth x Transect | 14 | 50903 | 3636 | 1.1029 | 0.263 | 997 |
| **Species x Depth x Transect** | **9** | **41599** | **4622** | **1.4021** | **0.028** | **997** |
| Residuals | 154 | 5.08E+05 | 3297 | | | |
| Total | 233 | 9.44E+05 | | | | |
| Pair-Wise Test: Species | | | | t | p(Perm) | Perm |
| *Lycodes adolfi, Lycodes polaris* | | | | 1.2345 | 0.153 | 998 |
| ***Lycodes adolfi, Lycodes sagittarius*** | | | | **1.8933** | **0.003** | **999** |
| ***Lycodes adolfi, Lycodes seminudus*** | | | | **1.7537** | **0.009** | **999** |
| ***Lycodes polaris, Lycodes sagittarius*** | | | | **1.8629** | **0.011** | **998** |
| *Lycodes polaris, Lycodes seminudus* | | | | 1.3569 | 0.120 | 999 |
| ***Lycodes sagittarius, Lycodes seminudus*** | | | | **2.7484** | **0.001** | **996** |
| Pair-Wise Test: Depth | | | | t | p(Perm) | Perm |
| **350, 1000** | | | | **1.894** | **0.002** | **999** |
| **500, 750** | | | | **1.881** | **0.006** | **997** |
| **500, 1000** | | | | **3.260** | **0.001** | **998** |
| **500, 1500** | | | | **1.696** | **0.047** | **998** |
| **750, 1000** | | | | **1.482** | **0.037** | **999** |
| **750, 1500** | | | | **1.532** | **0.028** | **998** |

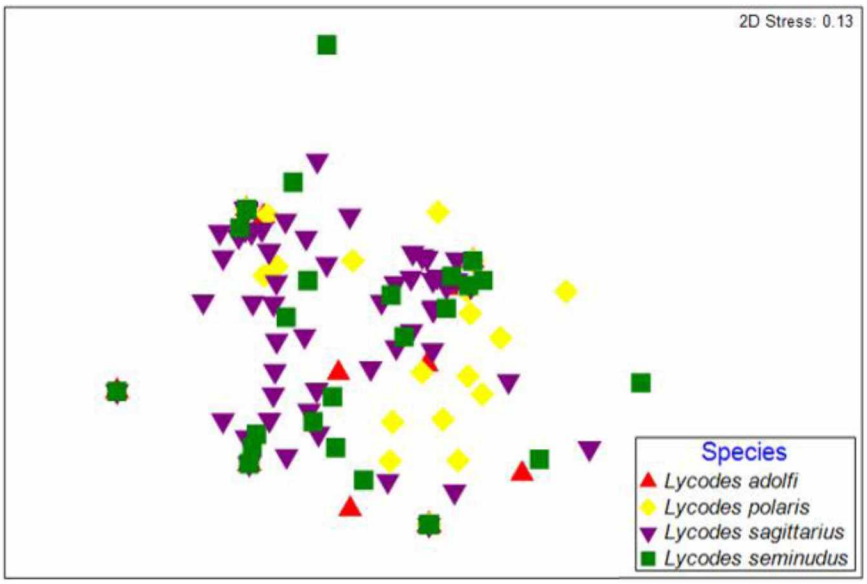


Figure 9. Non-metric multidimensional scaling (nMDS) plot of *Lycodes* spp. diet composition data by percent weight (%W) for central (2012) Beaufort Sea eelpouts. Each point represents one sample (stomach). Two outlier samples (*L. polaris, L. seminudus*) were excluded from the nMDS to better show distribution of remaining samples; outliers only contained 100% unidentified animal prey or teleost prey.

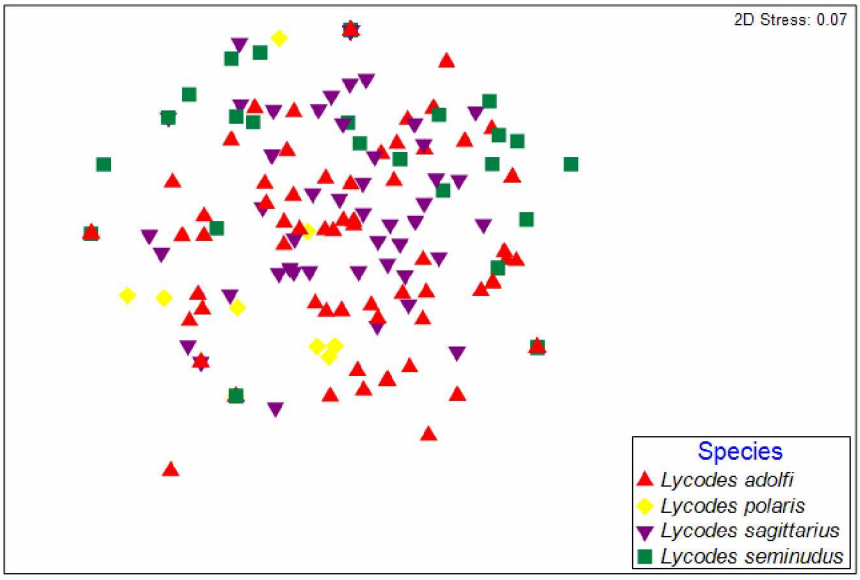


Figure 10. Non-metric multidimensional scaling (nMDS) plot of diet composition data by percent weight (%W) for eastern (2013/2014) Beaufort Sea eelpouts. Each point represents one sample (stomach). Two outlier *L. seminudus* stomachs that contained only teleost prey were excluded from the nMDS to better show distribution of remaining samples.

Similarity in diet composition within a species was low, 15% to 28.0% for 2012 fishes (Table 9), and 1.5% to 20% for 2013/2014 fishes (Table 10). Polychaeta and Crustacea were the main contributors to differences in diet composition for all pairings of eelpout species collected in 2012, while Polychaeta and Amphipoda were the main contributors to differences in diet composition among species for 2013/2014 fishes. In 2012, the prey group that contributed most to diet composition by %MW was unidentified Crustacea for *L. adolfi* (31%), Amphipoda for *L. polaris* (28%), and Polychaeta for both *L. sagittarius* (43%) and *L. seminudus* (27%) (Figure 11). In 2013 and 2014, Polychaeta was the most abundant prey group for *L. adolfi* (19%) and *L. sagittarius* (40%), Amphipoda for *L. polaris* (30%), and Ophiuroidea for *L. seminudus* (29%) (Figure 12).

Table 10. Percent mean weight (%MW) for coarse prey groups for eelpouts collected in central Beaufort Sea in 2012. In the upper section of the table average within group similarity (Avg. Sim.) is given for each eelpout species. Prey groups that together contributed at least 70% of the cumulative within-group similarity by %W are highlighted in bold, with the contributing percent similarity (%Sim.) indicated. The lower part of the table shows average between-eelpout species dissimilarity (Avg. Dis.) Prey groups that together contributed at least 70% of the cumulative percent dissimilarity are presented in descending order, followed by the cumulative percent dissimilarity (Cum. %) represented by those prey groups.

| | *L. adolfi* | | *L. polaris* | | *L. sagittarius* | | *L. seminudus* | |
|---|---|---|---|---|---|---|---|---|
| Avg. Sim. | | 14.6% | | 20.8% | | 28.0% | | 17.2% |
| Prey Group | %Sim. | (n=16) | %Sim. | (n=25) | %Sim. | (n=63) | %Sim. | (n=32) |
| Foraminifera | | 0.0 | | 0.0 | | 0.0 | | 0.0 |
| Mollusca | | 3.9 | | 0.0 | | 4.8 | | 4.3 |
| Polychaeta | | 12.6 | | 20.6 | 68% | **42.6** | 41% | **27.2** |
| Copepoda | | 16.2 | | 1.7 | | 4.1 | | 4.4 |
| Ostracoda | | 0.0 | | 0.2 | | 0.0 | | 0.4 |
| Cumacea | | 0.1 | 21% | **19.1** | | 2.4 | | 0.0 |
| Tanaidacea | | 0.0 | | 0.1 | | 0.3 | | 0.0 |
| Isopoda | | 14.1 | | 0.3 | | 12.7 | 26% | **21.1** |
| Amphipoda | 15% | **16.9** | 40% | **27.5** | 20% | **22.6** | 22% | **20.0** |
| Crustacea Unid. | 58% | **30.5** | 21% | **21.0** | | 7.2 | | 12.8 |
| Ophiuroidea | | 0.0 | | 4.0 | | 0.0 | | 2.9 |
| Teleost | | 0.0 | | 0.0 | | 0.7 | | 3.0 |
| Animal Unid. | | 0.0 | | 5.3 | | 0.0 | | 0.0 |
| Other | | 5.7 | | 0.3 | | 2.6 | | 3.9 |

| Between Sp. Dissimilarities | Avg. | Contributing prey groups | Cum. % |
|---|---|---|---|
| *L. sagittarius & L. seminudus* | 78.0 | Polychaeta, Amphipoda, Isopoda, Crustacea | 79.1 |
| *L. adolfi & L. sagittarius* | 84.7 | Polychaeta, Crustacea, Amphipoda, Isopoda | 77.4 |
| *L. adolfi & L. polaris* | 84.9 | Crustacea, Amphipoda, Polychaeta, Cumacea | 70.2 |
| *L. polaris & L. sagittarius* | 80.9 | Polychaeta, Amphipoda, Crustacea, Cumacea | 77.5 |
| *L. adolfi & L. seminudus* | 84.4 | Crustacea, Polychaeta, Amphipoda, Isopoda | 75.0 |
| *L. polaris & L. seminudus* | 84.7 | Polychaeta, Amphipoda, Crustacea, Isopoda | 71.2 |

Table 11. Percent mean weight (%MW) for coarse prey groups for eelpouts collected in eastern Beaufort Sea in 2013 and 2014. In the upper section of the table average within group similarity (Avg. Sim.) is given for each eelpout species. Prey groups that together contributed at least 70% of the cumulative within-group similarity by %W are highlighted in bold, with the contributing percent similarity (%Sim.) indicated. The lower part of the table shows average between-eelpout species dissimilarity (Avg.) Prey groups that together contributed at least 70% of the cumulative percent dissimilarity are presented in descending order, followed by the cumulative percent dissimilarity (Cum. %) represented by those prey groups.

| | | *L. adolfi* | | *L. polaris* | *L. sagittarius* | | *L. seminudus* | |
|---|---|---|---|---|---|---|---|---|
| Avg. Sim. | | 14.5% | | 16.7% | | 20.3% | | 15.3% |
| Prey Group | %Sim. | (n=107) | %Sim. | (n=9) | %Sim. | (n=67) | %Sim. | (n=50) |
| Foraminifera | | 3.5 | | 0.0 | | 1.8 | | 0.0 |
| Mollusca | | 3.0 | | 0.0 | 15% | **12.9** | | 0.0 |
| Polychaeta | 26% | **19.3** | | 21.6 | 60% | **40.3** | 26% | **25.6** |
| Copepoda | 23% | **15.7** | 12% | **15.1** | | 2.6 | | 5.0 |
| Ostracoda | | 2.7 | | 8.5 | | 3.1 | | 0.0 |
| Cumacea | | 3.4 | | 9.2 | | 3.5 | | 0.0 |
| Tanaidacea | | 6.1 | | 3.3 | | 8.2 | | 3.5 |
| Isopoda | 24% | **18.3** | | 0.0 | | 12.4 | | 12.9 |
| Amphipoda | | 12.1 | 69% | **30.3** | | 5.0 | | 6.8 |
| Crustacea Unid. | | 11.5 | | 12 | | 1.9 | | 7.7 |
| Ophiuroidea | | 0.0 | | 0.0 | | 3.5 | 52% | **28.9** |
| Teleost | | 0.0 | | 0.0 | | 0.0 | | 5.9 |
| Animal Unid. | | 3.6 | | 0.1 | | 0.9 | | 2.8 |
| Other | | 0.8 | | 0.0 | | 3.9 | | 0.9 |

| Between Sp. Dissimilarity | Avg. | Contributing prey groups | Cum.% |
|---|---|---|---|
| *L. sagittarius* & *L. seminudus* | 88.1 | Polychaeta, Ophiuroidea, Ispopoda, Mollusca, Amphipoda, Tanaidacea | 76.0 |
| *L. adolfi* & *L. sagittarius* | 86.1 | Polychaeta, Isopoda, Copepoda, Mollusca, Amphipoda, Crustacea | 74.6 |
| *L. adolfi* & *L. polaris* | 87.2 | Amphipoda, Polychaeta, Copepoda, Crustacea, Isopoda | 74.2 |
| *L. polaris* & *L. sagittarius* | 90.2 | Polychaeta, Amphipoda, Copepoda, Mollusca, Crustacea, Isopoda | 74.8 |
| *L. adolfi* & *L. seminudus* | 90.2 | Polychaeta, Ophiuroidea, Isopoda, Copepoda, Crustacea, Amphipoda | 78.3 |
| *L. polaris* & *L. seminudus* | 92.2 | Amphipoda, Ophiuroidea, Polychaeta, Copepoda, Crustacea | 71.3 |

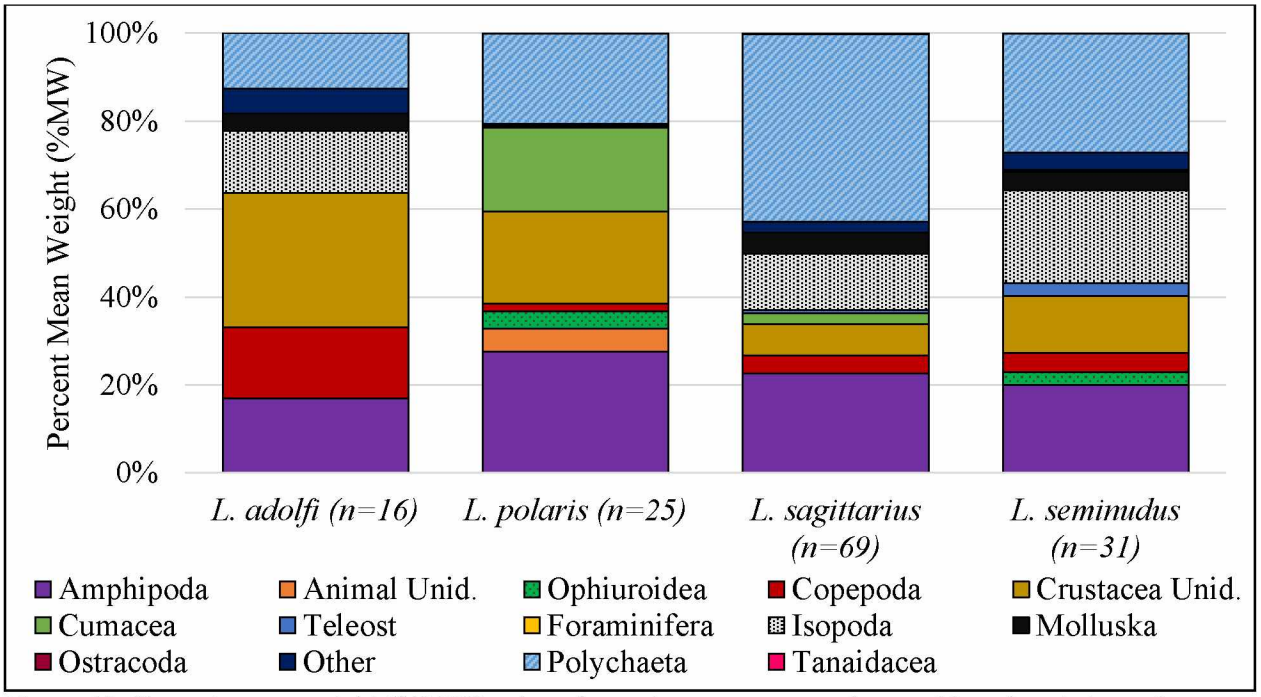


Figure 11. Percent mean weight (%MW) values for major prey groups observed in eelpout stomachs. Eelpouts were collected in the central Beaufort Sea (2012).

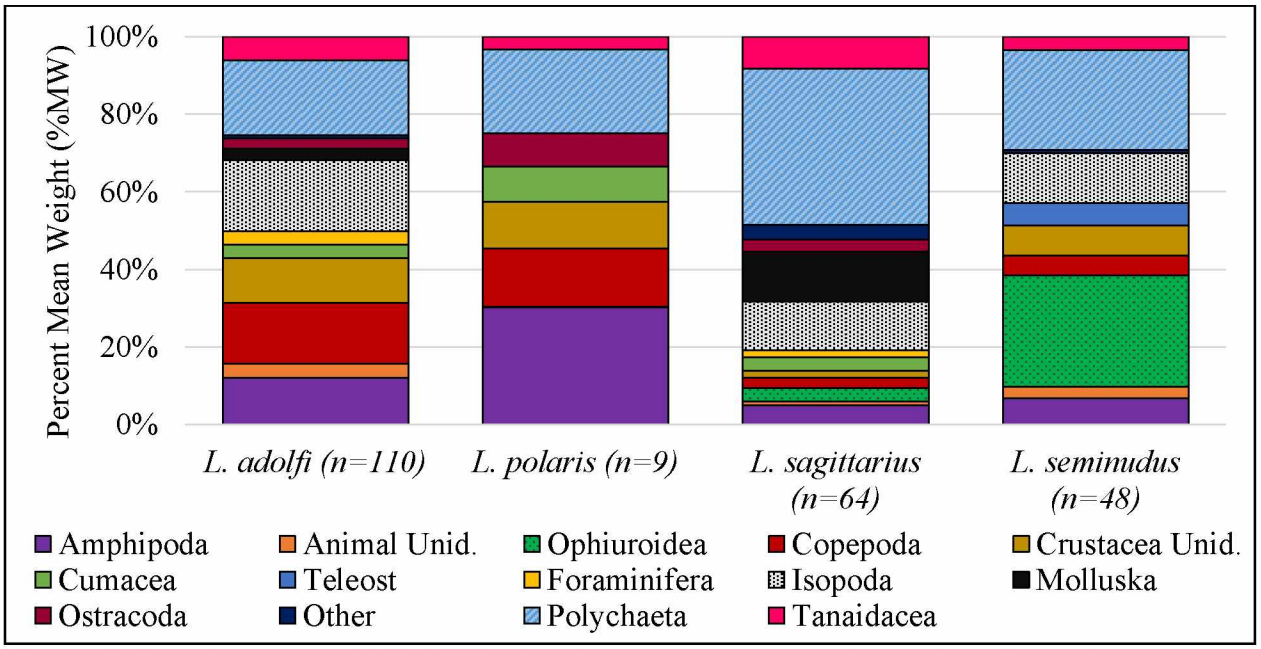


Figure 12. Percent mean weight (%MW) values for major prey groups observed in eelpout stomachs. Eelpouts were collected in the eastern Beaufort Sea (2013 and 2014).

An overall CCA permutation test indicated significant effects of environmental factors and fish length on the diets of *L. adolfi* and *L. sagittarius* (Table 11), but not on those of *L. polaris* and *L. seminudus*. After accounting for effects of all other variables by testing marginal effects, fish length was the only variable that had a significant effect on the diet composition of *L. adolfi* and *L. sagittarius*. (Table 12). Environmental variables (bottom temperature, depth, along shelf (i.e., longitude), and salinity) did not have significant effects when tested by marginal effects. Positioning of coarse prey groups in association with CCA axes indicated that Polychaeta was associated with increasing *L. adolfi* total length (Figure 13), while smaller *L. adolfi* were associated with Copepoda, Ostracoda, and Cumacea. For *L. sagittarius* unidentified animal tissue and Polychaeta were associated with increasing total fish length (Figure 14). Copepoda, Ostracoda, Tanaidacea, Isopoda, and unidentified Crustacea were all negatively associated with increasing total length.

Table 12. Results from overall permutation tests for canonical correspondence analysis (CCA) of the diet compositions (%W) of four eelpout species. Significant models ($\alpha = 0.05$) are indicated in bold font. The degrees of freedom (DF), chi square value (ChiSquare), F-value (F), and P-value (Pr(>F) are given for each test.

| | Df | ChiSquare | F | Pr(>F) |
|---|---|---|---|---|
| *L. adolfi* (n=107) | | | | |
| Model | 5 | 0.567 | 1.76 | **0.006** |
| Residual | 99 | 6.380 | | |
| *L. polaris* (n=9) | | | | |
| Model | 5 | 2.200 | 1.370 | 0.182 |
| Residual | 3 | 0.964 | | |
| *L. sagittarius* (n=67) | | | | |
| Model | 5 | 0.950 | 1.929 | **0.003** |
| Residual | 56 | 5.515 | | |
| *L. seminudus* (n=50) | | | | |
| Model | 5 | 1.046 | 1.434 | 0.071 |
| Residual | 45 | 6.342 | | |

Table 13. Permutation tests for marginal effects of terms and for each constrained axis from canonical correspondence analyses (CCA) of the diet composition (%W) of *L. adolfi* and *L. sagittarius*.

| ***L. adolfi*** | Df | ChiSquare | F | Pr(>F) | ***L. sagittarius*** | Df | ChiSquare | F | Pr(>F) |
|---|---|---|---|---|---|---|---|---|---|
| Fish Length | 1 | 0.257 | 3.981 | **0.001** | Fish Length | 1 | 0.316 | 3.207 | **0.001** |
| Temperature | 1 | 0.071 | 1.103 | 0.330 | Temperature | 1 | 0.119 | 1.207 | 0.254 |
| Salinity | 1 | 0.038 | 0.589 | 0.679 | Depth | 1 | 0.056 | 0.564 | 0.660 |
| Along Shelf | 1 | 0.086 | 1.332 | 0.202 | Along Shelf | 1 | 0.064 | 0.664 | 0.778 |
| Depth | 1 | 0.105 | 1.626 | 0.078 | Salinity | 1 | 0.095 | 0.964 | 0.489 |
| Residual | 99 | 6.3802 | | | Residual | 56 | 5.5149 | | |
| | | | | | | | | | |
| CCA1 | 1 | 0.308 | 4.774 | **0.001** | CCA1 | 1 | 0.550 | 5.585 | **0.001** |
| CCA2 | 1 | 0.117 | 1.809 | 0.060 | CCA2 | 1 | 0.180 | 1.823 | **0.029** |
| CCA3 | 1 | 0.084 | 1.302 | 0.232 | CCA3 | 1 | 0.152 | 1.539 | 0.151 |
| CCA4 | 1 | 0.046 | 0.719 | 0.679 | CCA4 | 1 | 0.043 | 0.438 | 0.907 |
| CCA5 | 1 | 0.013 | 0.197 | 0.995 | CCA5 | 1 | 0.026 | 0.262 | 0.992 |
| Residual | 99 | 6.3802 | | | Residual | 56 | 5.5149 | | |

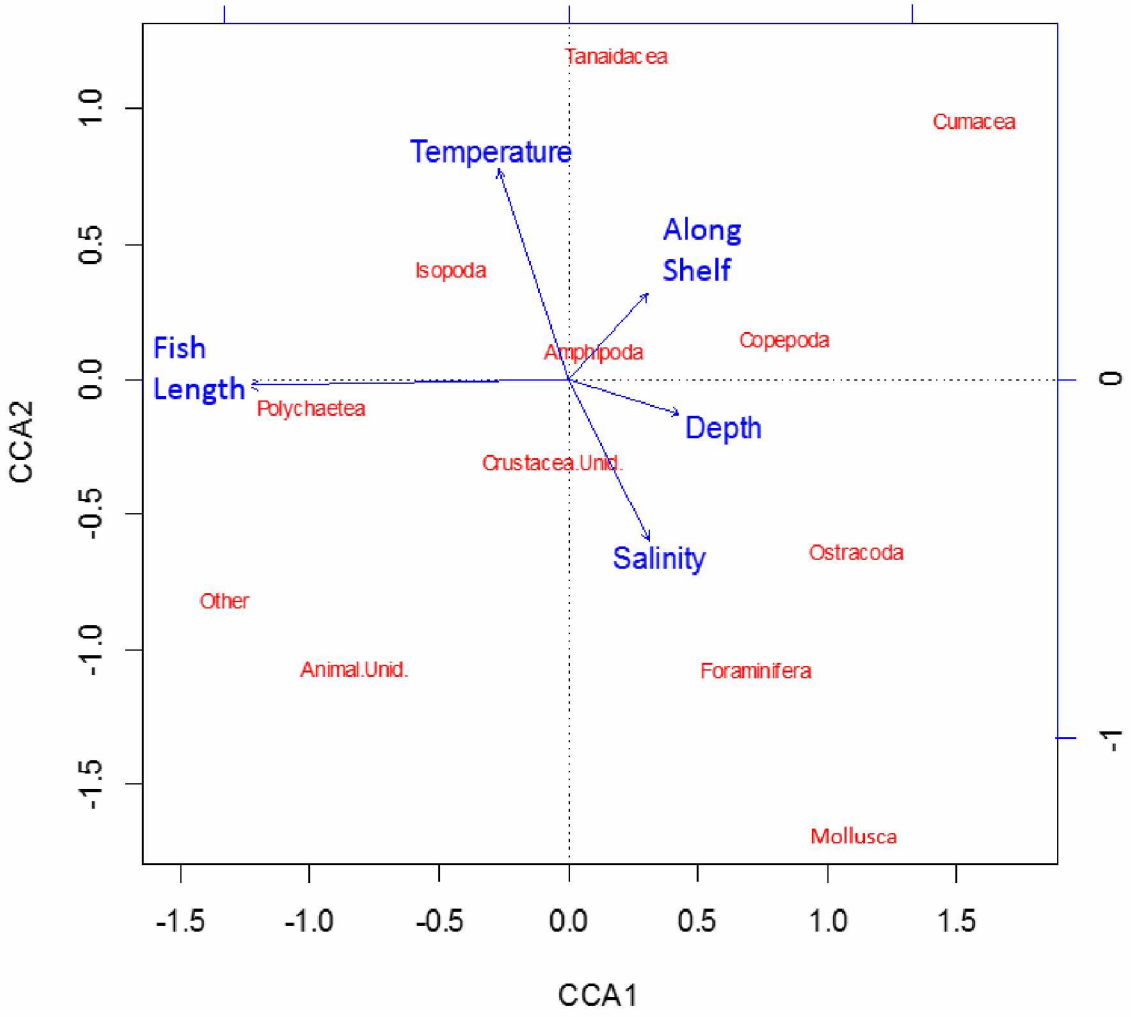


Figure 13. Canonical correspondence analysis (CCA) output for *L. adolfi* (2013 and 2014 sample years). The coarse prey groups (red) are multivariate response variables. Along-shelf (proxy for longitude), total fish length, temperature (°C), salinity (g/kg), and depth (m) are continuous factors (blue). The location of the mean responses of the coarse prey groups in relation to the continuous vectors is indicative of a prey group's association with a given environmental factor.

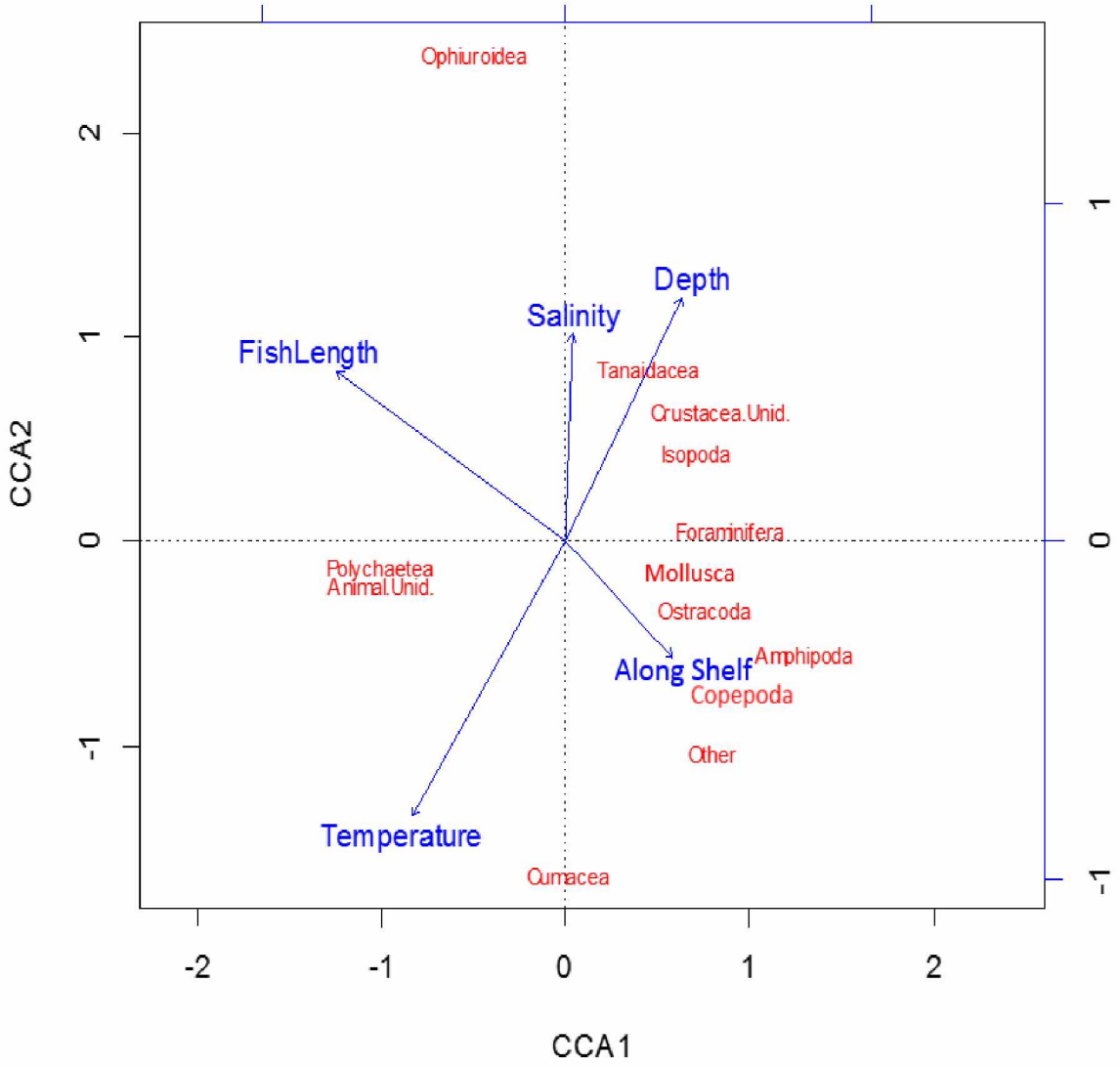


Figure 14. Canonical correspondence analysis (CCA) output for *L. sagittarius* (2013 and 2014 sample years). The coarse prey groups (red) are multivariate response variables. Along-shelf (proxy for longitude), total fish length, temperature (°C), salinity (g/kg) and depth (m) are continuous factors (blue). The location of the mean responses of the coarse prey groups in relation to the continuous vectors is indicative of a prey group's association with a given environmental factor.

*Size Class Analysis*

Size class analysis using nMDS indicated differences in diet with length for two of the three eelpout species examined. Sample size for *L. polaris* was inadequate for this analysis. At 40% similarity, *L. adolfi* partitioned into two main clusters of roughly fish ≤ 100 mm and fish ≥ 101 mm (Figure 15). However, some fish < 90 mm grouped into the ≥ 101 mm cluster and the size group 111 – 120 mm was an outlier. At 40% similarity *L. sagittarius* grouped into two main clusters: ≤ 150 mm and fish ≥ 151 mm, with a separate 101 – 130 mm group as an outlier (Figure 16). *Lycodes seminudus* did not cluster into continuous size groups.

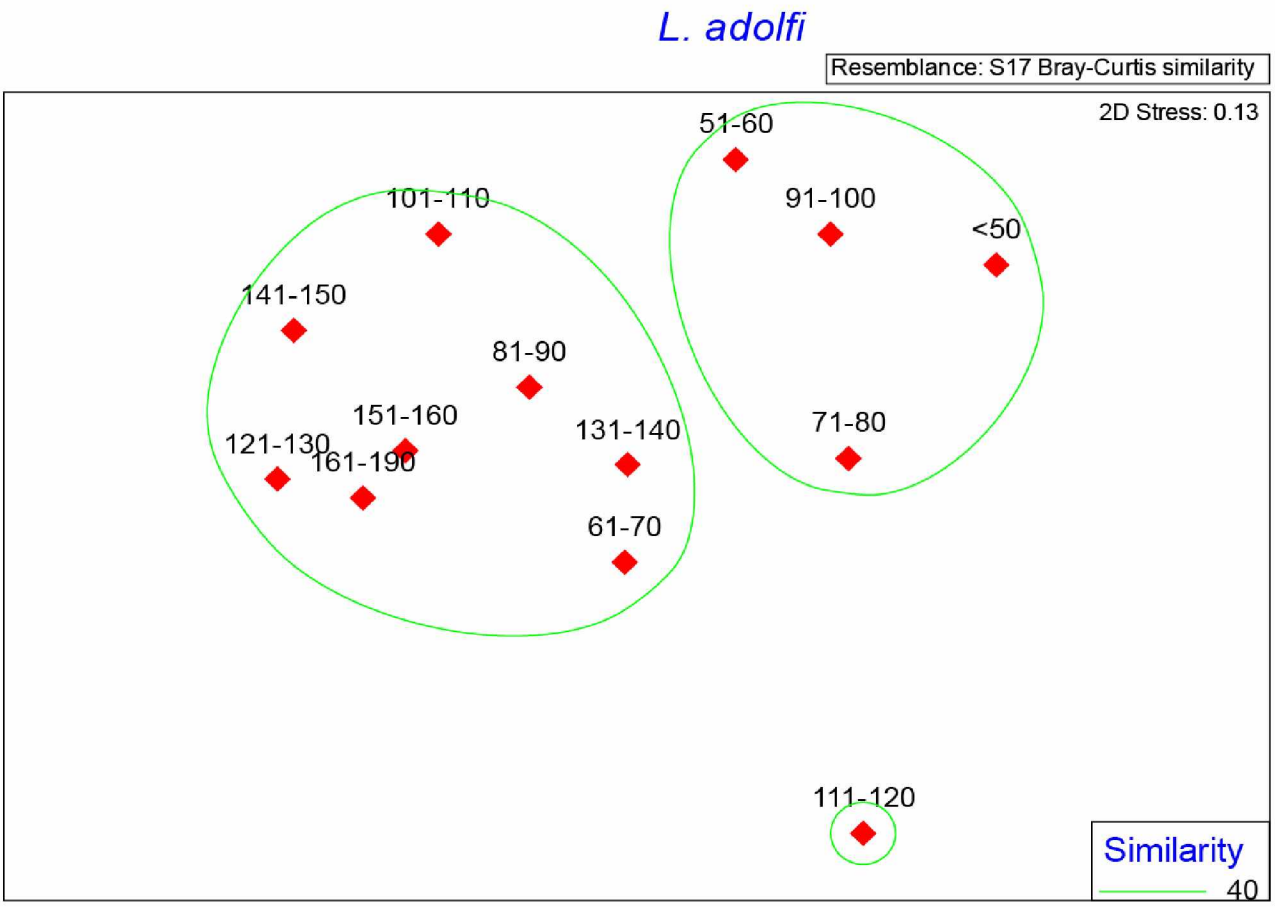


Figure 15. Similarity of *L. adolfi* prey composition by fish length bins. Percent similarity of 40% was used to detect consecutive sized groups of fish with similar diet composition.

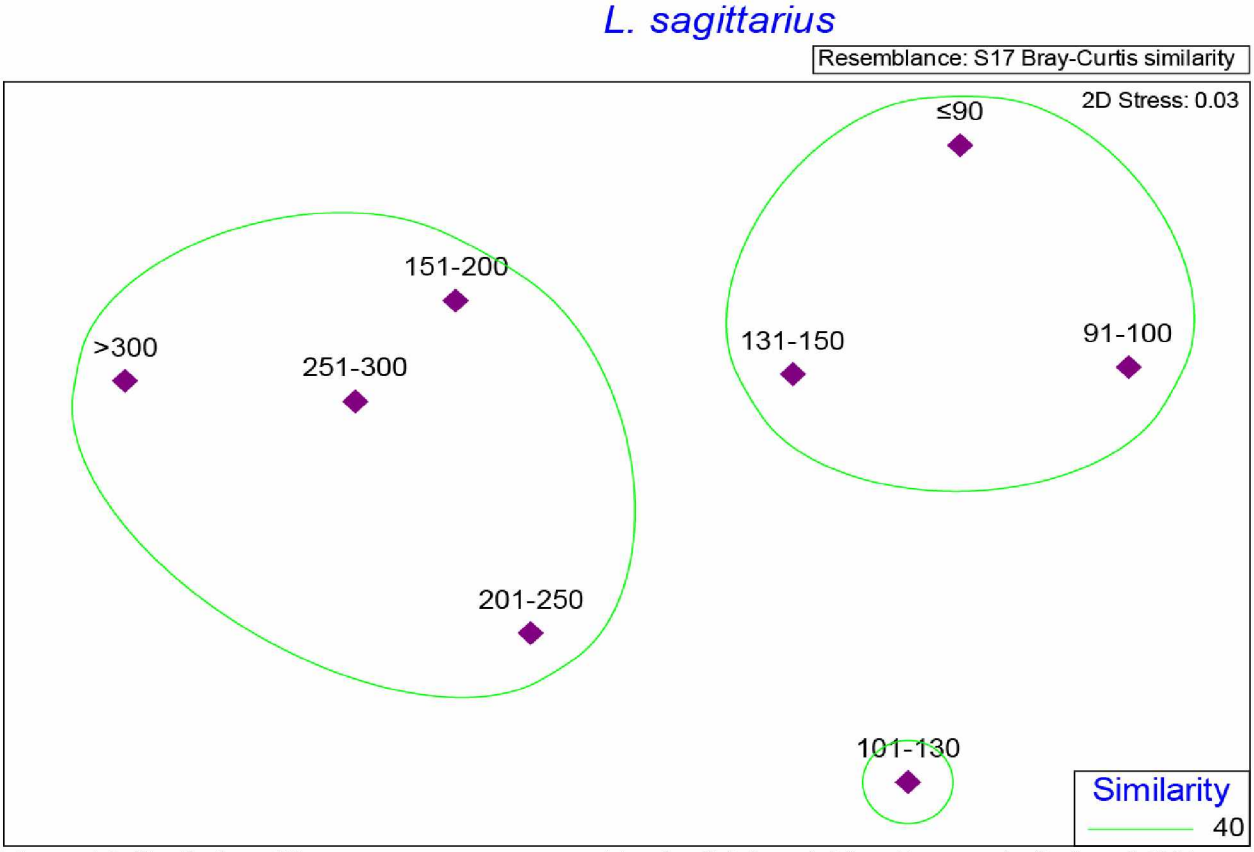

Figure 16. Similarity of *L. sagittarius* prey composition by fish length bins. Percent similarity of 40% was used to detect consecutive sized groups of fish with similar diet composition.

*Trophic Level and Carbon Sourcing*

Eelpout species differed significantly in their average nitrogen stable isotope values and the resulting trophic levels. The average nitrogen isotope value was lowest for *L. polaris* (15.0‰) and highest for *L. seminudus* (17.3‰) with intermediate values for *Lycodes adolfi* (17.0‰) and *L. sagittarius* (16.7‰). Nitrogen isotope values overlapped for the three deep-water species *L. adolfi, L. sagittarius*, and *L. seminudus,* but were significantly lower for *L. polaris* (Table 14, Figure 17). *Lycodes seminudus* and *L. sagittarius* also had significantly different $\delta^{15}N$ values despite considerable overlap. Similarly, calculated trophic levels (TL) overlapped for the three deep-water eelpouts, but were significantly lower for *L. polaris* (3.9 ± 0.2 SD, Table 15, Figure 18). TL was highest for *L. seminudus* (4.4 ± 0.4) and slightly lower (4.3 ± 0.3) for *L. adolfi* and *L. sagittarius*.

No significant differences in average carbon stable isotope values were detected among the four eelpout species ($F = 2.37$, $P = 0.072$), with large overlap in the ranges among species (Figure 17). This indicates

similar carbon sources in diets among the four eelpout species. Average $\delta^{13}C$ signatures ranged from -20.7‰ (*L. adolfi* and *L. polaris*) to -20.2‰ (*L. seminudus*)) with an intermediate value for *Lycodes sagittarius* (-20.5‰).

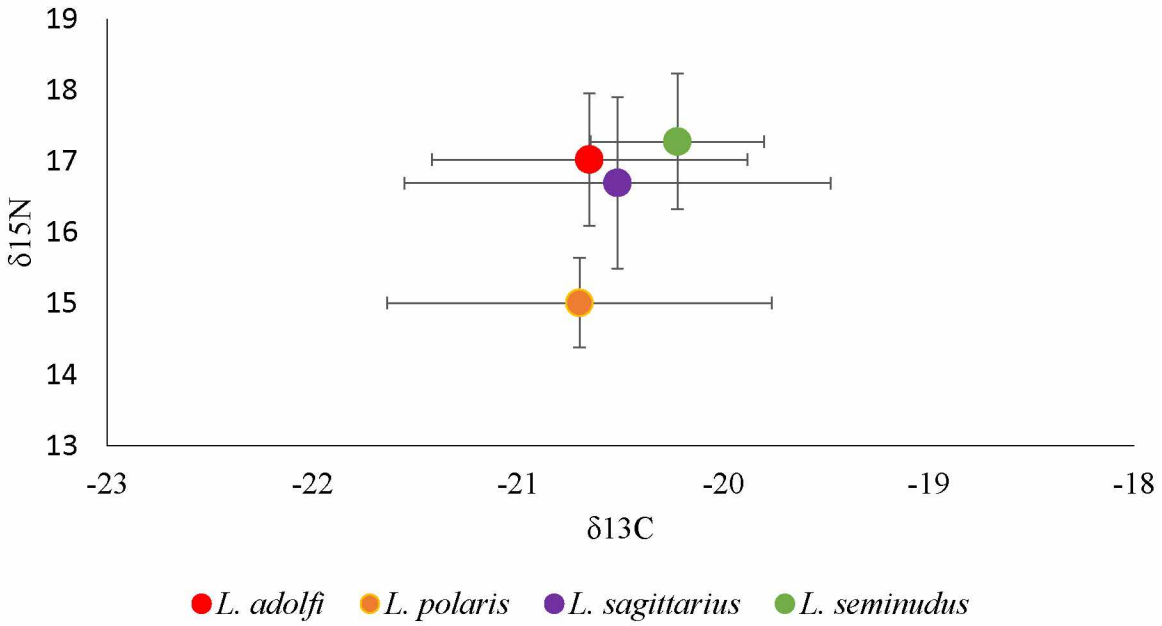


Figure 17. Stable nitrogen and carbon isotope values for four eelpout species. Dots are mean values and error bars are standard deviations. Fishes were collected in 2014.

Table 14. One-way ANOVA of differences between $\delta^{15}N$ and between $\delta^{13}C$ values for four eelpout species in 2014. Non-significant (NS) tests are indicated. Sums of squares (SS), degrees of freedom (df), mean squares (MS), the calculated F statistic (F), and the critical F statistic (F crit). Subsequent pairwise test were conducted using Dunn's method and gave a q-value (q).

| **ANOVA $\delta^{15}N$** | | | | | | |
|---|---|---|---|---|---|---|
| Source of Variation | SS | df | MS | F | p | F crit |
| Between Species | 73.8 | 3 | 24.6 | 7.074 | **0.0002** | 2.652 |
| Within Species | 667.8 | 192 | 3.5 | | | |
| Total | 741.6 | 195 | | | | |
| **ANOVA $\delta^{13}C$** | | | | | | |
| Source of Variation | SS | df | MS | F | P-value | F crit |
| Between Species | 4.8 | 3 | 1.6 | 2.371 | 0.072 | 2.654 |
| Within Species | 123.9 | 184 | 0.7 | | | |
| Total | 128.7 | 187 | | | | |
| **Pairwise Test for $\delta^{15}N$** | | | | **Diff of Ranks** | **q** | **P<0.05** |
| *L. polaris* vs. *L. seminudus* | | | | 113.0 | 6.592 | **P<0.05** |
| *L. polaris* vs. *L. adolfi* | | | | 95.0 | 6.081 | **P<0.05** |
| *L. polaris* vs. *L. sagittarius* | | | | 75.9 | 4.706 | **P<0.05** |
| *L. seminudus* vs. *L. sagittarius* | | | | 37.1 | 3.101 | **P<0.05** |
| *L. adolfi* vs. *L. sagittarius* | | | | 19.1 | 1.974 | NS |
| *L. seminudus* vs. *L. adolfi* | | | | 18.1 | 1.601 | NS |

Table 15. One-way ANOVA and pairwise test of rank-based calculated trophic level for the four eelpout species. Only 2014 fish were used for this analysis. Subsequent pairwise test were conducted using Dunn's method and gave a q-value (q).

| **ANOVA Trophic Level** | | | | | | |
|---|---|---|---|---|---|---|
| Source of Variation | SS | df | MS | F | P-value | F crit |
| Between Species | 3.67 | 3 | 1.22 | 15.09 | **<0.0001** | 2.651 |
| Within Species | 15.71 | 194 | 0.08 | | | |
| Total | 19.38 | 197 | | | | |
| **Pairwise Test for Trophic Level** | | | | **Diff of Ranks** | **Q** | **P<0.05** |
| *L. polaris* vs. *L. sagittarius* | | | | 77.32 | 4.80 | **P<0.05** |
| *L. polaris* vs. *L. seminudus* | | | | 92.29 | 5.38 | **P<0.05** |
| *L. polaris* vs. *L adolfi* | | | | 87.96 | 5.63 | **P<0.05** |
| *L. seminudus* vs. *L. sagittarius* | | | | 14.97 | 1.25 | NS |
| *L. adolfi* vs. *L. sagittarius* | | | | 10.64 | 1.10 | NS |
| *L. seminudus* vs. *L. adolfi* | | | | 4.33 | 0.38 | NS |

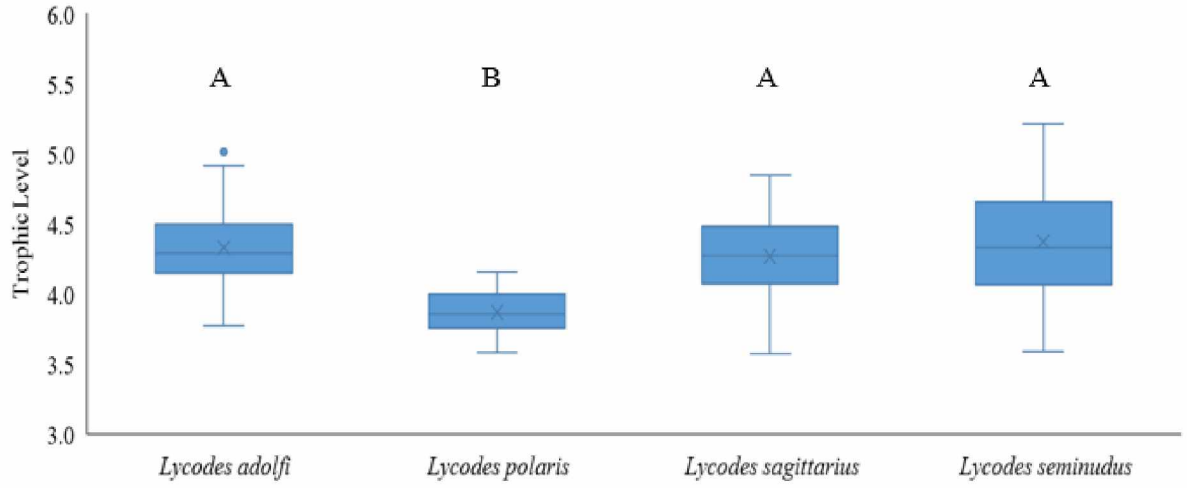


Figure 18. Calculated trophic levels for *L. adolfi* (n=85), *L. polaris* (n=16), *L. sagittarius* (n=60), and *L. seminudus* (n=37). The median is indicated with an **X** and average is indicated by a horizontal line. The first and fourth quartiles are represented by the vertical lines. Outliers are represented with individual dots. Species designated with the letter **A** were significantly different from species designated with **B**.

The relationship between increasing fish length and stable isotope value (TL or $\delta^{13}C$) was curvilinear for eelpout species in which a significant relationship existed. The best fit model suggested a curved rather than linear relationship with TL (Table 16 and Figure 19) and increasing fish length. In addition, this curved relationship was different for each of the four eelpout species and was most pronounced for *L. adolfi* and *L. polaris*, which had lower TL values at intermediate sizes. The relationship for *L. sagittarius* was not significant. This could be due to smaller sample size and less statistical power. Similar to TL, carbon stable isotope also had a curvilinear relationship with increasing fish length (Table 17 and Figure 20), but the relationship was not significant for *L. polaris* or *L. seminudus*. Similar to TL, the carbon values for *L. adolfi* were lowest at intermediate values.

Table 16. Results of comparison of multiple analysis of covariance (ANCOVA) for trophic level and fish length. The presence and number of asterisk indicates the degree of statistical significance. A full ANCOVA for best model by AIC is given. Asterisk (*) indicates significance of linear, one degree polynomial (poly(Length, 2)1), or two degree polynomial (poly(Length, 2)2) term.

| Comparison of ANCOVA Models for Trophic Level (TL) | | | | | |
|---|---|---|---|---|---|
| | AIC | Model | | | |
| Model 1 | 66.4 | Differences among species, no length effect | | | |
| Model 2 | 64.8 | Linear model, single slope across species | | | |
| Model 3 | 53.4 | Quadratic model, same shape for each species | | | |
| Model 4 | 71.1 | Linear model, different line for each species | | | |
| Model 5 | 20.0 | Quadratic model, different shape for each species | | | |
| ANCOVA for Model 5 | | | | | |
| | Estimate | Std. Error | t value | Pr(>\|t\|) | |
| Intercept | 5.0 | 0.12 | 40.79 | $<2x10^{-16}$ | *** |
| *L. polaris* | 2.3 | 1.76 | 1.30 | 0.1950 | |
| *L. sagittarius* | -0.8 | 0.13 | -6.18 | $4.43x10^{-9}$ | *** |
| *L. seminudus* | -0.7 | 0.13 | --5.23 | $4.69x10^{-7}$ | *** |
| *L. adolfi* poly(Length, 2)1 | 23.2 | 3.90 | 5.94 | $5.94x10^{-8}$ | *** |
| *L. polaris* poly(Length, 2)1 | 92.6 | 45.26 | 2.05 | 0.0423 | * |
| *L. sagittarius* poly(Length, 2)1 | 0.7 | 0.46 | 1.52 | 0.1308 | |
| *L. seminudus* poly(Length, 2)1 | -0.8 | 0.49 | -1.54 | 0.1247 | |
| *L. adolfi* poly(Length, 2)2 | 14.9 | 2.75 | 5.42 | $1.80x10^{-7}$ | *** |
| *L. polaris* poly(Length, 2)2 | 35.9 | 20.07 | 1.79 | 0.0756 | * |
| *L. sagittarius* poly(Length, 2)2 | -0.01 | 0.67 | -0.02 | 0.9853 | |
| *L. seminudus* poly(Length, 2)2 | 2.0 | 0.41 | 4.91 | $1.95x10^{-6}$ | *** |

Table 17. Results of comparison of multiple analysis of variance (ANCOVA) for $\delta^{13}C$ and fish length. The presence and number of asterisk indicates the degree of statistical significance. A full ANCOVA for the best model by AIC is given. Asterisk (*) indicates significance of linear, one degree polynomial (poly(Length, 2)1), or two degree polynomial (poly(Length, 2)2) term.

| Comparison of ANCOVA Models for $\delta^{13}C$ | | | | | |
|---|---|---|---|---|---|
| | AIC | Model | | | |
| Model 1 | 465.5 | Differences among species, no length effect | | | |
| Model 2 | 449.0 | Linear model, single slope across species | | | |
| Model 3 | 450.9 | Quadratic model, same shape for each species | | | |
| Model 4 | 442.3 | Linear model, different line for each species | | | |
| Model 5 | 432.1 | Quadratic model, different shape for each species | | | |
| ANCOVA for Model 5 | | | | | |
| | Estimate | Std. Error | t value | Pr(>\|t\|) | |
| Intercept | -19.2 | 0.37 | -51.59 | $2.0 \times 10^{-16}$ | *** |
| *L. polaris* | -5.5 | 5.25 | -1.04 | 0.29847 | |
| *L. sagittarius* | -1.6 | 0.39 | -4.15 | $5.21 \times 10^{-5}$ | *** |
| *L. seminudus* | -1.2 | 0.39 | -2.98 | 0.00329 | ** |
| *L. adolfi* poly(Length, 2)1 | 49.9 | 11.67 | 4.27 | $3.18 \times 10^{-5}$ | *** |
| *L. polaris* poly(Length, 2)1 | -103.8 | 135.4 | -0.77 | 0.44425 | |
| *L. sagittarius* poly(Length, 2)1 | 8.0 | 1.37 | 5.88 | $1.95 \times 10^{-8}$ | *** |
| *L. seminudus* poly(Length, 2)1 | 1.0 | 1.46 | 0.71 | 0.47849 | |
| *L. adolfi* poly(Length, 2)2 | 32.5 | 7.67 | 4.23 | $3.59 \times 10^{-5}$ | *** |
| *L. polaris* poly(Length, 2)2 | -46.7 | 59.6 | -0.79 | 0.3366 | |
| *L. sagittarius* poly(Length, 2)2 | 1.2 | 0.56 | 0.56 | 0.5694 | |
| *L. seminudus* poly(Length, 2)2 | 0.5 | 1.19 | 0.44 | 0.6327 | |

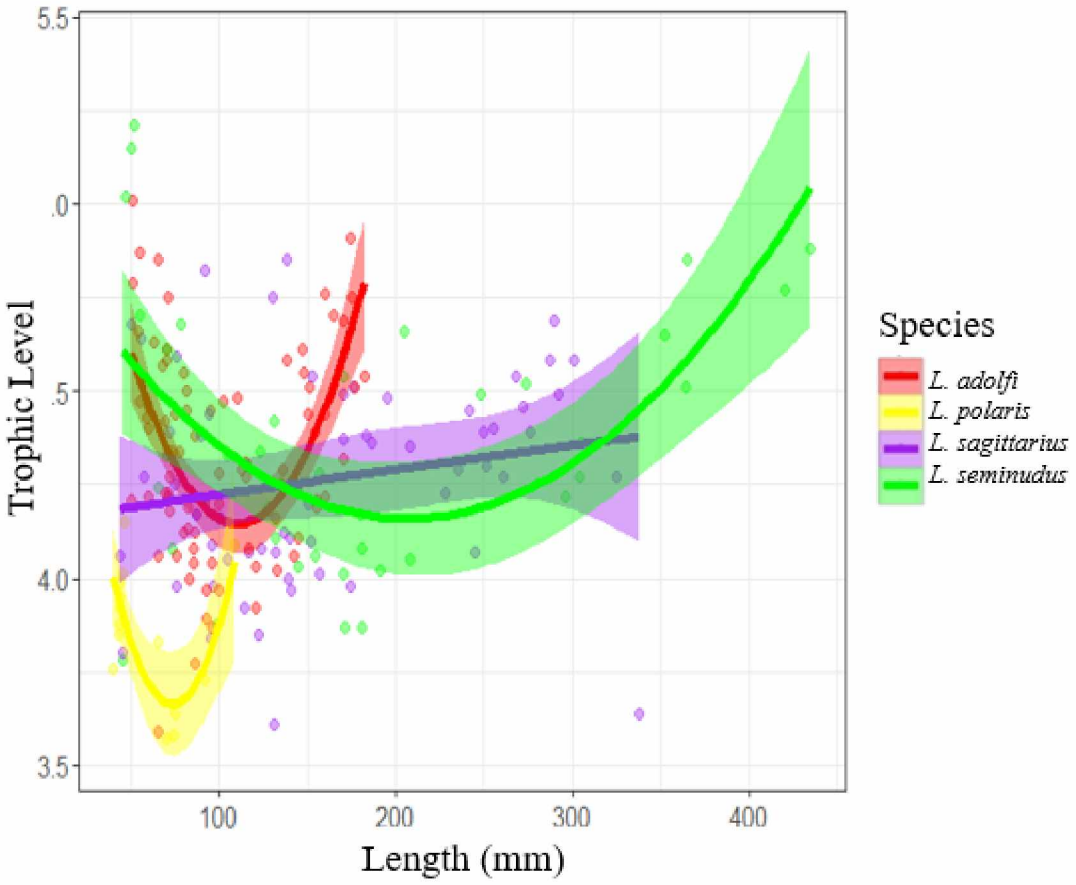


Figure 19. Trophic level (TL) against length for each of the four eelpout species. The model of best fit from the ANCOVA is shown as selected based on AIC.

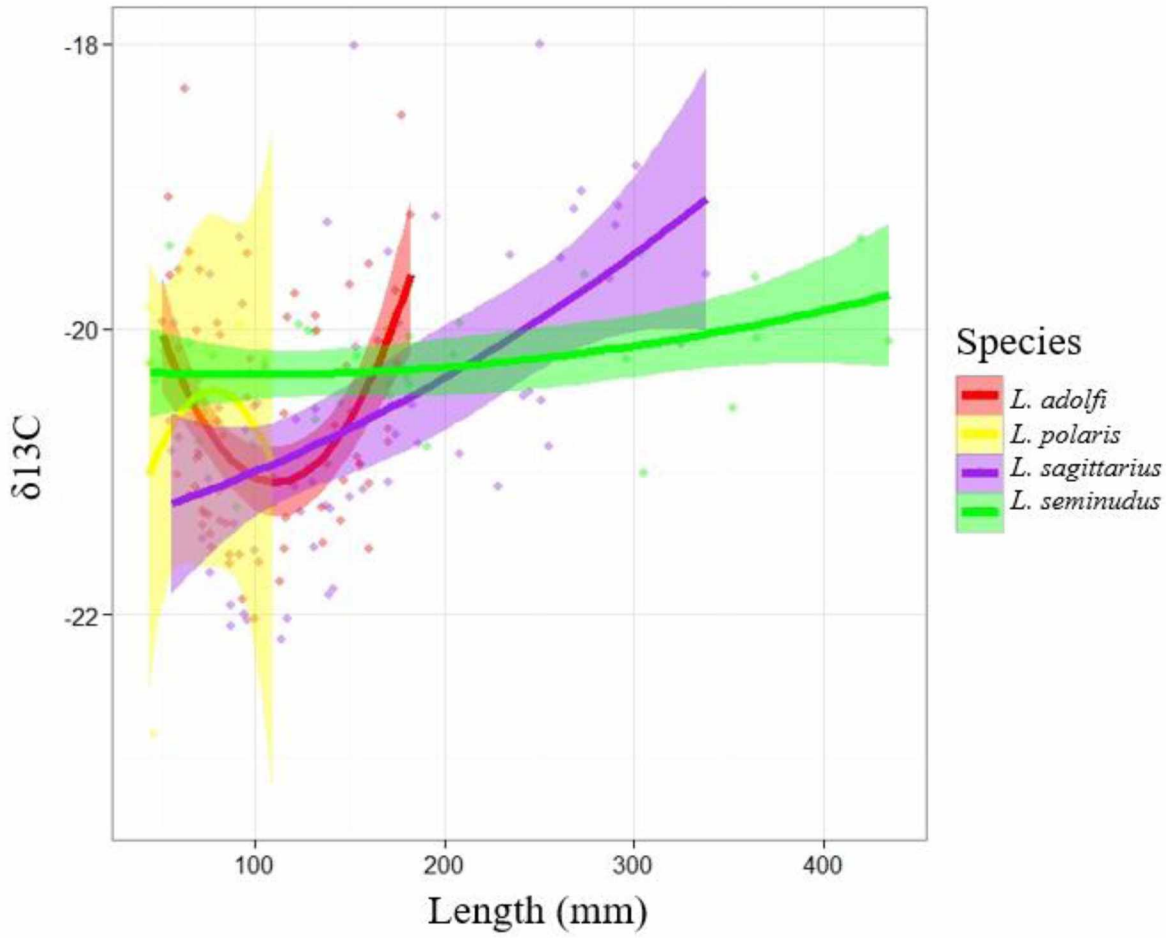


Figure 20. Carbon stable isotope ($\delta^{13}C$) against length for each of the four eelpout species. The model of best fit from the ANCOVA is shown as selected based on AIC.

## Discussion

This study describes previously unknown diet composition for four eelpout species found in the U.S. Beaufort Sea: *L. adolfi*, *L. polaris*, *L. sagittarius*, and *L. seminudus*. Eelpout diets were dominated by demersal prey such as polychaetes, amphipods, isopods, and brittle stars. The prevalence of demersal prey in eelpout diet is consistent with previous studies of *Lycodes* diet in the Beaufort Sea (Dissen 2015; Giraldo et al. 2016), Chukchi Sea (Whitehouse et al. 2017), and Atlantic Arctic (Bjelland et al. 2000). A shift from benthic-directed energy flow to primarily pelagic food webs is expected to occur in the Arctic due to climate warming, and is already thought to be occurring in the nearby northern Bering Sea (Grebmeier et al. 2006). If the predicted shift from a benthic dominant to pelagic dominant food web occurs, eelpout dependence upon demersal prey may leave them susceptible to energy shortages due to decreased benthic prey and could result in smaller eelpout population sizes. Fish length and habitat depth were significant predictors of diet composition. Depth correlated with eelpout species distribution, with *L. polaris* being found primarily on the shelf and *L. adolfi*, *L. sagittarius*, and *L. seminudus* found on the slope. Diet composition based on stomach contents differed among eelpout species, but the observed patterns varied based on location of sampling. For example, in the central Beaufort Sea diet was different between *L. polaris* and *L. adolfi*, and *L. polaris* and *L. sagittarius*. No significant difference in diet composition was observed between all other eelpout species pairings in the central Beaufort. The opposite was true in the eastern Beaufort Sea where diets differed significantly between all pairings of eelpout species, the exception being *L. polaris* and *L. adolfi*, and *L. polaris* and *L. seminudus*. Average stable nitrogen isotope values, as a measure of eelpout diet and TL over a longer time scale than stomach content analysis (months vs. hours or days, respectively), indicated that the shelf species *L. polaris* fed at a lower TL than the three slope species. Average overall carbon isotope values were not significantly different among the four eelpout species despite sampling fishes from across a wide longitudinal range representative of differing conditions due to varying terrestrial and freshwater matter influences. This indicates similar basal carbon sources for all four eelpout species. Stable isotope values had a curvilinear relationship with increasing fish length, indicating TL changes over ontogeny in non-linear ways for some species. This study provides a detailed look into eelpout diet that is valuable for further understanding of the role and vulnerability of this genus in the current Arctic ecosystem.

### *Species Identification*

Genetic testing used in this study for eelpout species identification indicated potential problems with the currently accepted taxonomy of *Lycodes*. Eelpout identification and current taxonomy is primarily based

on morphology (Møller and Gravlund 2003) but a high degree of phenotypic plasticity is known to exist for some eelpout species (Balanov and Kukhlevskii 2011). Accuracy of identification based on genetics ranged from 68% to 89% for the four eelpout species included in this study. The difficulty to accurately identify species based on morphology observed in the present study confirmed that the four eelpout species may exhibit high phenotypic plasticity. Additionally, there also may be very little genomic differentiation between some of the currently accepted Arctic eelpout species, and some of these species may in fact represent synonyms. Genetic differentiation between *L. polaris* and *L. seminudus* was especially low (< 1.4%). For marine teleosts, average within-species variability is generally 0.39% (minimum 0%; maximum 14.08% Kimura 2-parameter (K2P) percent), average across-genus variability is 9.93% (minimum 0%; maximum 20.63%), and 15.46% across-family (minimum 1.39%; maximum 35.72%) (Ward et al. 2005). DNA sequence divergence of up to 1-2% is generally accepted within a single species (Ward et al. 2009). This suggests that individuals identified as *L. polaris* or *L. seminudus* may be closely related members of the same complex of currently recognized species. By itself, mtDNA is not enough to justify grouping these two species as one. However, the genetic closeness of *L. polaris* and *L. seminudus* means any interspecific differences in diet or TL between these two species may not be due to well defined species differences, and instead may be driven by differences in fish size or distribution with depth.

*Inter- and intraspecific diet differences*

The four eelpout species across both the central and the eastern Beaufort Sea had diets composed primarily of benthic prey reflective of their demersal habits. Diet information based on stomach contents for these four species in this region was absent before this study, but findings on diet composition are consistent with studies in neighboring regions. In the Chukchi Sea, gammarid amphipods comprise a significant proportion of the diet of *L. polaris* (Whitehouse et al. 2017), similar to the Beaufort Sea. Amphipods of the family Oedicerotidae, a family of gammaridean amphipods, were found in both Chukchi Sea and Beaufort Sea *L. polaris* stomachs, suggesting this amphipod family is an important prey source for *L. polaris* across a broad geographic range. Stable isotope and fatty acid analyses characterized *L. polaris* as a low-TL benthic generalist in the neighboring Canadian Arctic (Giraldo et al. 2016). *Lycodes adolfi* in the Canadian Arctic consume crustaceans and are benthic generalists (Coad and Reist 2004; Giraldo et al. 2016). Similar to Canadian Arctic *L. adolfi*, Beaufort Sea *L. adolfi* consumed benthic prey, with a high proportion of crustacean prey, but also Polychaeta (13% MW in 2012 and 19% MW in 2013/2014). Both *L. sagittarius* and *L. seminudus* in the Canadian Arctic consume polychaetes and

crustaceans (Coad and Reist 2004), similar to those from Beaufort Sea studied here. Benthic feeding habits of *L. seminudus* in the Beaufort Sea also were confirmed by fatty acid analyses (Dissen 2015). The prevalence of benthic prey in diets of the four eelpout species agrees with studies of eelpout diets in neighboring seas, emphasizing the general importance of benthic prey to the diet of this genus.

In both the central and the eastern Beaufort Sea, *L. polaris* and *L. seminudus* diet compositions did not differ significantly from each other. Also, little genetic distance between *L. polaris* and *L. seminudus* was observed in the species confirmation section of this study. Low genetic distance, along with similar diet contents, may indicate that *L. polaris* and *L. seminudus* are recently diverged species. However, incorporating stable isotope biomarkers weakens this conclusion. Similar diets may indicate similar TL (Parish 1975), and based on stomach contents alone, it could be assumed that the two eelpout species are feeding across the same TL. However, in this study there were significant differences in TL between *L. polaris* and *L. seminudus*. The observed lack of significant difference in diet composition could be an artifact of the limited 'snapshot' time period represented by stomach contents versus longer-term biomarkers like stable isotopes and fatty acids. Stomach content analysis can also be biased towards hard bodied prey. Soft bodied prey are digested more quickly and are often only identifiable by residual body parts (e.g., polychaete chaetae), biasing stomach content analysis results and resulting in discrepancies with biomarker results (i.e., stable isotopes) (Weidner et al. 2017). Lastly, low sample numbers for *L. polaris* likely impacted power of statistical tests. *Lycodes polaris* and *L. seminudus* from the central and eastern Beaufort Sea have been shown to have differing diets (Dissen 2015), though both relied heavily on demersal prey. Subtle differences in diet composition were likely lost due to low sample sizes and high intraspecies variability.

Diet composition of all four eelpout species was driven by differences in the relative contributions of the same few prey groups. These prey groups: Polychaeta, Isopoda, Copepoda, Amphipoda, and Mollusca, were comprised of diverse, and primarily benthic associated prey items. Brittle stars (Ophiuroidea) were important, but only in the diet of *L. seminudus*. Polychaeta were particularly important for the two larger, deep-water eelpout species *L. sagittarius* and *L. seminudus*. The prevalence of Polychaeta and dominance of demersal prey groups in eelpout diet mirrors characteristics of the Arctic invertebrate community. Polychaeta, along with Mollusca, Amphipoda, and Echinodermata are the most numerous invertebrate groups in the Beaufort and neighboring seas (Rand and Logerwell 2011; Blanchard et al. 2013; Ravelo et al. 2015). Eelpout diets could be a reflection of spatial patterns in prey availability. For example, direct comparison of diet composition of snow crabs (*Chionoecetes opilio*) to prey populations across the

Beaufort and Chukchi seas indicated a lack of prey selection, and, therefore, crab diet was driven by patterns in prey distribution and availability (Divine et al. 2015). Such a comparative analysis of eelpout stomach contents to patterns in invertebrate populations was not possible for this study. Relevant prey samples were not available to make these comparisons.

Nitrogen stable isotope values for the four eelpout species indicated that *L. polaris* occupies a lower TL than the other three eelpout species, all of which fed at the same TL. Amphipoda was the dominant prey group in *L. polaris* diet (27% in 2012 and 30% in 2013/2014) based on stomach content analysis, but was also present in the three other eelpout species (ranging from 17% to 28% in 2012 and 5% to 12% in 2013/2014 for the three other eelpout species). Amphipoda is a trophically diverse group, including herbivores, carnivores, scavengers, or some combination of feeding types (Poltermann 2001; Arndt et al. 2005). The lower TL observed for *L. polaris* could be the result of consuming a higher proportion of lower TL amphipods than the other three eelpout species. However, the family Oedicerotidae observed in *L. polaris* diet is generally carnivorous (Guerra-Garcia et al. 2014). It could be that *L. polaris* is consuming additional lower TL amphipods not represented in the stomach content analysis, or which are obscured in the unidentified Amphipoda group. Cumacea also was an important prey group, having a high %MW, for *L. polaris* (19% in 2012 and 9% in 2013/2014), but not the other three eelpout species (0 – 2% in 2012 and 0 – 4% in 2013/2014). The Cumacea *Diastylis* spp. found in *L. polaris* is a benthic surface deposit feeder and is characterized by a very low TL (TL of 1.6 to 0.4, Bell et al. 2016). The presence of Cumacea in *L. polaris* diet, and the absence of Cumacea in the diet of the other three eelpout species, could be driving the observed difference in TL between *L. polaris* and the other three eelpout species. Lastly, as the stable isotope values represent diet integrated over a longer time period than stomach contents (Sakano et al. 2005; Weidel et al. 2011), the differences in diets between *L. polaris* and the other three eelpout species seem to be a persistent feature.

Average TL alone indicates that *L. adolfi*, *L. sagittarius*, and *L. seminudus* are feeding at the same TL, and therefore, these three eelpout species could be competing for similar resources. Competition for resources can occur among fish species that occupy the same habitat and TL (Parish 1975). Alternatively, the lack of significant differences in TL among the three deep-water eelpout species may be because they are consuming different prey, but prey that have similar TLs. Stomach content analysis indicated Polychaeta were the top prey item for the three deep-water eelpout species. Each eelpout species consumed different polychaete families, but *Lycodes sagittarius* consume a more diverse array of polychaete families (e.g., Lumbrineridae, Maldanidae, Nephtyidae, Opheliidae, Paraonidae, Spionidae)

than *L. adolfi* or *L. seminudus* (mostly Polynoidae, Lumbrineridae, and Nephtyidae). Polychaeta is a species rich group and their ecology is diverse. Of those families observed in eelpout stomachs, Lumbrineridae, Nephtyidae, and Polynoidae are carnivores, while Maldanidae consume detritus (Fauchald and Jumars 1979). Trophic levels of Arctic polychaetes reflect the ecological diversity of the group, with estimated TL ranges reflective of primary consumers (TL = 1) to top predators (TL = 4) (Iken et al. 2005; Bell et al. 2016). Differences in time represented by stable isotope analysis versus stomach contents may also account for the discrepancy in the results for the three deep-water eelpouts. The lack of differences in TL among the three deep-water eelpouts could indicate that over a longer time scale these three species consume similar prey, and that differences observed in diet from stomach contents are only representative of the specific sampling time. In this study, $\delta^{15}N$ and TL had a curvilinear relationship with increasing fish length, meaning eelpouts shift trophic levels with increasing length. TLs generally increase with increasing fish length (Marsh et al. 2012) due to greater gape size (Scharf et al. 2000) and expansion in foraging range. Intermediate length *L. seminudus* and *L. adolfi* exhibited lower trophic level. Decreasing TL with length has been observed for Capelin *Mallotus villosus* in the Chukchi Sea (Marsh et al. 2012), but the non-linear relationship between TL and exhibited by the two eelpout species is unusual. One possible explanation is that small eelpouts may consume small, but high TL prey (e.g., *Anonyx* sp., TL: 2.4 – 3.5, Bell 2015), shift to large but low TL prey (e.g., *Ophiocten sericeum*, TL: 2.0) at intermediate sizes, and large high trophic prey (e.g., teleost *Boreogadus saida*, TL: 2.7 – 3.8), thus driving the observed pattern. Alternatively, TL may reflect available prey community composition at different habitat requirements at different life stages. Lastly, previous community-wide analyses using nitrogen and carbon stable isotopes of the Beaufort Sea ecosystem indicated that *L. adolfi* and *L. seminudus* were top TL predators within the fish community (Bell et al. 2016), and these findings are supported by the high trophic levels found in the present study.

The high intraspecific dissimilarity in diet composition, along with the high number of different prey items found in eelpout diets, may be indicative of generalist feeding for *L. adolfi* and *L. seminudus*. Generalists feed on a broad array of prey compared with specialists that may only feed on a few prey types. *Lycodes adolfi* and *L. seminudus* had the lowest average percent intraspecific similarity of diet composition of the four eelpout species, meaning they exhibit a relatively higher degree of generalist feeding, and they had high trophic levels. This is consistent with other studies in the adjacent Canadian Beaufort Sea that classified *L. adolfi* and *L. seminudus* as mid- to high-TL generalist feeders (Giraldo et al. 2016). *Lycodes polaris* and *L. sagittarius*, though having diverse diets, show some partial diet

preferences or specialization. *Lycodes polaris* has sometimes been classified both as a generalist in the Canadian Beaufort Sea (Giraldo et al. 2016), and a semi-specialist consumer, primarily of gammarid amphipods in the eastern Chukchi Sea (Whitehouse et al. 2017). My study found that amphipods, the vast majority of which were gammarid amphipods, composed 28 – 30% of *L. polaris* diet, suggesting some degree of specialization. Comparing diet over a broad geographic scale (e.g., across seas) is valuable as individuals of a species can exhibit localized specialization, while the population on a whole is generalist (Fox and Marrow 1981). Given gammarid amphipods are important for both *L. polaris* in the Chukchi Sea (Whitehouse et al. 2017) and Beaufort Sea (this study), evidence suggest *L. polaris* is a specialist. *Lycodes sagittarius* may also be a semi-specialist feeder. The presence of vomerine teeth indicates that *L. sagittarius* may specialize in crushing large, hard shelled prey (McAllister et al. 1981). Mollusca were an important prey group for *L. sagittarius* in the present study (4.8 – 12.9%), and with the relatively high within-group similarity, may suggest specialization. Other Arctic demersal fishes like sculpins and flatfishes have also been designated as generalists (Gray et al. 2017; Whitehouse et al. 2017). A generalist approach to feeding may be advantageous in a dynamic and ever-changing ecosystem like the Arctic (Chambers and Dick 2005) because it likely allows switching to prey sources that may become more abundant.

*Across- and along-shelf influences*

Across-shelf changes (i.e., depth) were significant predictors of all eelpout species' diet composition. Depth is a proxy for changes in water masses and food supply conditions, which drive patterns of epifauna and infauna community composition in the Beaufort Sea (Nephin et al. 2014; Ravelo et al. 2015; Roy et al. 2015). Depth coincides with changes in benthic invertebrate community composition and abundance, with greatest abundance observed at the shelf break from 50 to 100 m (Iken et al. 2016). Patterns in availability of potential eelpout prey with depth could be contributing to the observed differences in diet composition for eelpouts.

Carbon stable isotope signature is indicative of basal carbon source of a food chain and, in this particular system, also of across-shelf distance based on influence of terrestrial vs. marine derived carbon sources (Romanuk et al. 2011; Dunton et al. 2012; Bell et al. 2016). Though no differences in average $\delta^{13}C$ existed among the four eelpout species, $\delta^{13}C$ did change with increasing fish length (i.e., curvilinear relationship). While average carbon isotope values for all four eelpout species were similar, ranging from -22.84‰ for *L. polaris* to -22.03‰ for *L. adolfi*. Using cornerstone values of -24.0 ± 0.4‰ for particulate

organic matter (POM) from marine phytoplankton, -21.6 ± 0.5‰ for ice associated production, and -28.8 ±3.2‰ for terrestrial matter (Dunton et al. 2012, Bell et al. 2016), the curvilinear relationship may indicate that differently sized eelpouts are a member of energy paths that build on different basal carbon sources. Eelpout length is influenced by depth, with larger fishes occupying greater depths, and, therefore, the observed increase in $\delta^{13}C$ values with length may be due to increasing distance offshore of larger fish, meaning their diet is more based on a marine carbon source. High $\delta^{13}C$ observed for the smallest *L. adolfi* sampled may indicate marine carbon sourced prey, and midsized *L. adolfi* is based more heavily on terrestrial sourced carbon than smaller or larger eelpouts.

Along-shelf changes (i.e., longitude) in diet composition were significant or not depending on region sampled. Along-shelf was not significant for eastern Beaufort Sea (2013 and 2014) fishes, but was significant for central Beaufort Sea (2012) fishes based on PERMANOVA. The differences in significance of longitude and eelpout diet are reflective of larger scale patterns in benthic invertebrate communities. Longitudinal patterns occur in benthic invertebrate communities in the western and central Beaufort Sea (Ravelo et al. 2015). These same along-shelf invertebrate community patterns could be reflected in eelpout diet across the central Beaufort Sea sampling area. The eastern Beaufort Sea is heavily influenced by organic matter input from the Mackenzie River (Bell et al. 2016). The vast influence of the Makenzie River plume may result in a more homogeneous benthic invertebrate population, and benthic invertebrate biomass and abundance do not have strong longitudinal trends in the eastern Beaufort Sea (Iken et al. 2016). The lack of strong along-shelf changes in invertebrate patterns is reflected in eelpout diet for the 2013 and 2014 sampling area. The lack of along-shelf diet differences was reflected in carbon isotope values in this study. Carbon isotope signatures indicate basal carbon source of an organism's diet (i.e., terrestrial vs. pelagic or sea ice associated production) (Iken et al. 2005; Gradinger 2009; Bell et al. 2016). Enrichment of stable carbon isotope signature with increasing TL is minimal, conserving basal carbon source signatures in higher TL consumers (Romanuk et al. 2011). The absence of a significant difference in carbon stable isotope values among eelpouts further supports that, at least in the eastern Beaufort Sea, there are no along-shelf differences in diet. Elsewhere, spatial differences in prey species distribution and composition drive diet composition of predatory fish (Hovde et al. 2002; Jaworski and Ragnarsson 2006). In the Arctic, along-shelf spatial variation in fish diet has been observed for Arctic Cod *Boreogadus* saida (Gray et al. 2017), and the invertebrate predator snow crab *Chionoecetes opilio* (Divine et al. 2015). For those eelpout species that exhibit generalist patterns in

feeding, like *L. adolfi* and *L. seminudus*, eelpout diet is reflective of along-shelf homogeneous patterns in prey composition for the eastern Beaufort Sea.

*Eelpout Morphology*

Individual fish length was an important factor in determining composition of eelpout diets, and may contribute to limiting resource competition. Length is a factor in diet for other Arctic fish species like Arctic Cod *Boreogadus saida* (Gray et al. 2016) and sculpins in the Beaufort Sea (Gray et al. 2017), and a possible mechanism for avoidance of competition. In the present study, length was particularly important for determining diet composition for *L. adolfi* and *L. sagittarius*. For large eelpouts like *L. sagittarius*, larger total size increases mobility (Scharf et al. 2000), which is advantageous in deep habitats where prey can be scarce. Biomass and abundance of epibenthic biomass decrease with increasing depth in the eastern Beaufort Sea (Iken et al. 2016). *Lycodes adolfi* do not have the advantage of large size, and may use other means, like targeting different prey types, to thrive on the slope. *Lycodes adolfi* and *L. sagittarius* could compete for resources due to their overlapping distributions, but *L. adolfi* may exploit different sized prey than the larger *L. seminudus*. *Lycodes polaris* and *L. adolfi* exhibit similar size ranges, but *L. polaris* is on the shelf and *L. adolfi* is on the slope; potential interspecies competition is likely avoided by minimizing overlap in species distributions.

Fish length is positively related to fish gape size (Scharf et al. 2000), and as expected, this pattern was observed for the four eelpout species in this study. Though gape height and fish length were linearly related for all species, eelpout species had different gape sizes at the same length. *Lycodes seminudus* had the largest average gape size at a given length, followed by *L. sagittarius*, *L. adolfi*, and *L. polaris*. This has important ecological implications, because as gape height increases the size range of prey that can be consumed increases (Scharf et al. 2000). If this holds true for eelpouts, then a 300 mm *L. seminudus* should be able to consume larger size range of prey than a 300 mm *L. sagittarius*. Likewise, *L. adolfi* and *L. polaris* only reach maximum sizes of approximately 200 mm in length and, therefore, would not be capable of consuming the largest potential prey of 400 mm *L. sagittarius* or *L. seminudus* due to their relative smaller length and corresponding gape size. Though the size range of prey did increase with increasing fish length, multiple prey that appeared to be larger than maximum gape height were consumed. It should be noted that in this study prey length was measured, and not prey width, resulting in long prey like polychaetes having a disproportionate influence on the relationship between predator length and gape width. Not only does size of prey differ with increasing fish length, composition of prey

also changed with increasing fish length. In this study, prey groups associated with greater fish length were polychaetes, brittle stars, and isopods. These were some of the largest sized prey observed. Large (> 240 mm) *L. seminudus* consumed large fish (total length; all ≥58 mm). In contrast, smaller eelpouts of all four species consumed small prey such as harpacticoid copepods, small cumaceans, and tanaids. Significant difference in diet composition between large (≥151 mm) and small (≤150 mm) *L. sagittarius* was supported from the cluster analysis. The difference in the type and size of prey consumed between large and small fish indicates an ontogenetic shift in diet. Intraspecific competition for resources is potentially minimized by smaller eelpouts consuming different types and sizes of prey than larger eelpouts in part because of differences in gape size at length. Likewise, interspecific competition is minimized by eelpouts of similar sizes, but different species, having differing gape sizes and therefore utilizing different prey.

Stomachs of all *Lycodes* species often contained highly digested prey items or were empty. This was observed in both small and large eelpouts. This may be due to small gape size and less mobility in small eelpouts, low metabolic needs associated with slow growth and cold temperatures, which reduce metabolic needs for larger eelpouts. Low growth rates, even when compared with other zoarcids, have been observed for Arctic *Lycodes* spp. (Hildebrandt et al. 2011). Long periods between feeding likely result in more stomachs with unidentifiable, heavily digested prey contents or empty stomachs. High numbers of empty stomachs have hindered previous attempts at characterizing diets of *L. seminudus* and other Arctic *Lycodes* in the eastern Norwegian Sea, including *L. frigidus, L. pallidus, L. eudipleurostictus*, and *L. esmarki* (Bjelland et al. 2000). Approximately 21% of *Lycodes* stomachs were excluded from the present study because they were empty or only contained parasites. Though empty stomachs provide information on the proportion of empty and full stomachs, they do not provide information on fish diet composition in studies that use only stomach contents. Using biomarkers like stable isotope or fatty acid signatures in conjunction with stomach content analyses, as in this study, should be considered when studying diet of this genus, as they are not reliant on having full stomachs.

*Conclusions*

Eelpout diet composition is diverse and composed primarily of benthic prey. Competition for resources among eelpouts is reduced by fishes of different species and lengths inhabiting different depths, and different eelpout species consuming different amounts of certain prey types due at least in part to differences in gape size. Stomach contents and stable isotope analyses used in this study provide

information on diet and trophic ecology over different time scales. Using both methods provides an in-depth examination of eelpout diet ecology over a portion of the Beaufort Sea. *Lycodes* spp. are one part of the Arctic food web at trophic levels 3.9 – 4.4, and, like other fish species in the region, connect lower and upper trophic levels. They are consumed by other animals such as Greenland shark, bearded seals, and various seabirds (Finley and Evans 1983; Antonelis et al. 1994) and by Greenland shark in the north Atlantic (Yano et al. 2007). They may also serve as potential competitors with other fish species for prey resources and space. For example, *L. polaris* diet overlaps with Arctic Staghorn Sculpin *Gymnocanthus tricuspis* (Giraldo et al. 2016). These two species have similar spatial distributions in the central and western Beaufort Sea (Mecklenburg et al. 2011; Norcross et al. 2015), and, therefore, likely compete for prey. Understanding trophic ecology of Arctic marine species like eelpouts is becoming more important as managers and major agencies are moving towards ecosystem-based management practices that require an in-depth knowledge of all abundant species, not just those with commercial or cultural importance (Chambers and Dick 2005; Källgren et al. 2015). Additionally, climate change is expected to shift the main energy pathways from the benthos to the pelagic zone (Grebmeier et al. 2006). Eelpouts feed heavily on benthic organisms and could be disproportionately affected by a shift from a benthic to a more pelagic dominated food web.

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# Appendix A
# 2013 IACUC #134765-12 Approval

(907) 474-7800
(907) 474-5638 fax
fyiacuc@uaf.edu
www.uaf.edu/iacuc

**Institutional Animal Care and Use Committee**
909 N Koyukuk Dr. Suite 212, P.O. Box 757270, Fairbanks, Alaska 99775-7270

June 21, 2012

To: Brenda Norcross, Ph.D.
Principal Investigator

From: University of Alaska Fairbanks IACUC

Re: [134765-10] Offshore fisheries surveys in the Chukchi and Beaufort Seas

The IACUC reviewed and approved the Amendment/Modification to the protocol referenced above by Designated Member Review.

| | |
|---|---|
| Received: | May 31, 2012 |
| Approval Date: | June 21, 2012 |
| Initial Approval Date: | December 18, 2007 |
| Expiration Date: | December 18, 2012 |

This action is included on the June 21, 2012 IACUC Agenda.

*The PI is responsible for acquiring and maintaining all necessary permits and permissions prior to beginning work on this protocol. Failure to obtain or maintain valid permits is considered a violation of an IACUC protocol, and could result in revocation of IACUC approval.*

*The PI is responsible for ensuring animal research personnel are aware of the reporting procedures on the following page.*

**Appendix B**
**2014 IACUC #134765-13 Approval**

(907) 474-7800
(907) 474-5638 fax
fyiacuc@uaf.edu
www.uaf.edu/iacuc

Institutional Animal Care and Use Committee
909 N Koyukuk Dr. Suite 212, P.O. Box 757270, Fairbanks, Alaska 99775-7270

January 11, 2013

To: Brenda Norcross, Ph.D.
Principal Investigator

From: University of Alaska Fairbanks IACUC

Re: [134765-13] Offshore fisheries surveys in the Chukchi and Beaufort Seas

The IACUC has reviewed the Progress Report by Designated Member Review and the Protocol has been approved for an additional year.

| | |
|---|---|
| Received: | December 10, 2012 |
| Initial Approval Date: | December 18, 2007 |
| Effective Date: | January 10, 2013 |
| Expiration Date: | December 18, 2013 |

This action is included on the January 24, 2013 IACUC Agenda.

*If you have any questions about how to submit the required information through IRBNet please contact the Office of Research Integrity for assistance (email fyori@uaf.edu or call x7800/x7832).*

# Appendix C
# 2015 IACUC #134765-14 approval

(907) 474-7800
(907) 474-5993 fax
uaf-iacuc@alaska.edu
www.uaf.edu/iacuc

**Institutional Animal Care and Use Committee**
909 N Koyukuk Dr. Suite 212, P.O. Box 757270, Fairbanks, Alaska 99775-7270

December 20, 2013

To: Brenda Norcross, Ph.D.
Principal Investigator

From: University of Alaska Fairbanks IACUC

Re: [134765-14] Offshore fisheries surveys in the Chukchi and Beaufort Seas

The IACUC has reviewed the Progress Report by Full Committee Review and the Protocol has been approved for an additional year.

| | |
|---|---|
| Received: | December 10, 2013 |
| Initial Approval Date: | December 18, 2007 |
| Effective Date: | December 19, 2013 |
| Expiration Date: | December 18, 2014 |

This action is included on the December 19, 2013 IACUC Agenda.

***PI responsibilities:***

- *Acquire and maintain all necessary permits and permissions prior to beginning work on this protocol. Failure to obtain or maintain valid permits is considered a violation of an IACUC protocol and could result in revocation of IACUC approval.*
- *Ensure the protocol is up-to-date and submit modifications to the IACUC when necessary (see form 006 "Significant changes requiring IACUC review" in the IRBNet Forms and Templates)*
- *Inform research personnel that only activities described in the approved IACUC protocol can be performed. Ensure personnel have been appropriately trained to perform their duties.*
- *Be aware of status of other packages in IRBNet; this approval only applies to this package and the documents it contains; it does not imply approval for other revisions or renewals you may have submitted to the IACUC previously.*
- *Ensure animal research personnel are aware of the reporting procedures detailed in the form 005 "Reporting Concerns".*